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TRANSFUSION

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The Curtain Rises

THE appearance of *yet another* journal is hardly expected to be greeted with universal acclaim. Statements such as "I can't even keep up with the existing ones" or "There is too much unnecessary publication now" are predictable. My own reaction, when it was originally suggested that the American Association of Blood Banks have an official scientific journal, was even more violently negative. Why did I change my mind?

The pressure of the need for an outlet for papers in this field was a major factor. The phenomenal growth of interest in blood groups, blood preservation, and general transfusion problems and the logarithmic increase of research has resulted in a greater flow of manuscripts than can adequately be handled by existing channels. The long jam of papers has caused annoying delays in their publication and has resulted in a demand for more rapid processing.

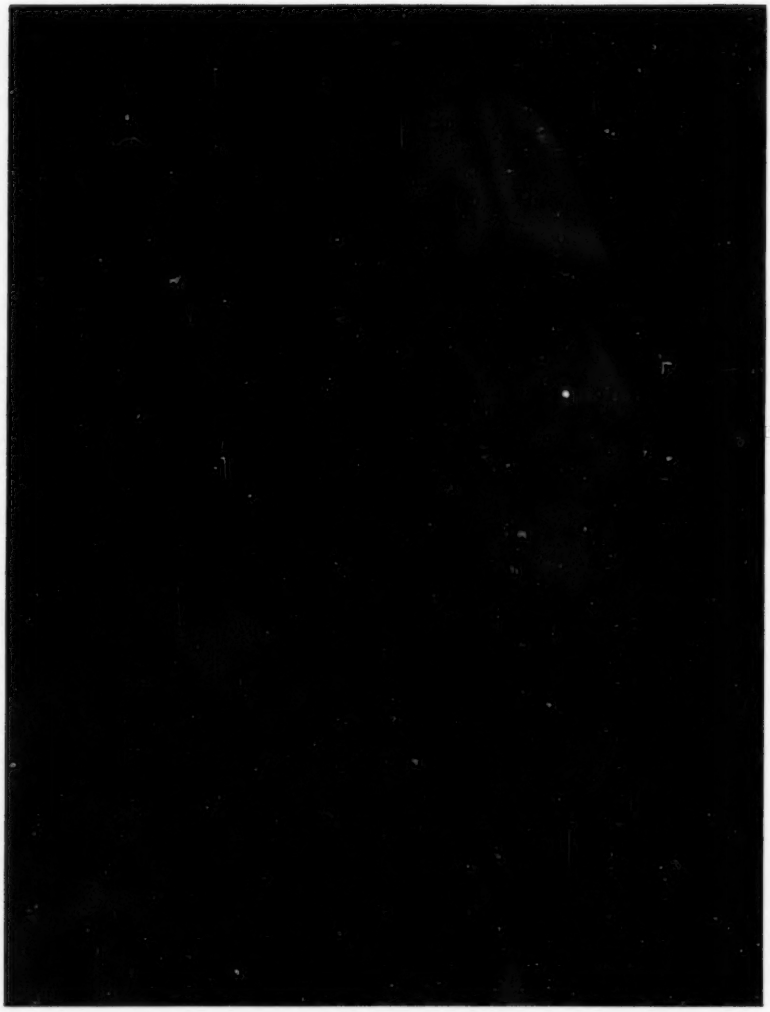
The lack of a sufficient number of journals specializing in the transfusion sciences has caused this material to become hopelessly dispersed in a large number and variety of periodicals making it practically impossible for anyone to maintain a broad purview.

We hope to attain these goals by publishing original papers dealing with all phases of clinical transfusion problems, technics, blood preservation, and blood-group immunology, genetics and anthropology.

Delay in publication is usually blamed on the editorial staff but authors are as much at fault. TRANSFUSION will favor manuscripts of 2,000 words or less (about eight double-spaced typewritten pages). Bibliographies should be restricted to pertinent references. Exhaustive reviews of the literature will be discouraged except in articles written for this purpose. Lengthier articles will be considered on the basis of merit and timeliness. Speed of publication can be maintained only if essayists follow the instructions to authors meticulously and strive for excellence in grammatical structure and style of presentation.

The editorial staff promises to discriminate against everyone regardless of previous reputation or present exalted status and will return, for revision by the authors, manuscripts which do not meet the necessary high standards.

Speedier publication of preliminary observations and briefly documented data will be encouraged in the form of short communications to the editor. Technical and administrative material will also be included. Abstracts, book reviews, questions and answers and Association and other news will make TRANSFUSION a publication essential to anyone whether his interest is limited to blood or to blood banking.



KARL LANDSTEINER
(Courtesy of A. S. Wiener, M.D.)

Memories of Karl Landsteiner

PHILIP LEVINE, M.D.

ASIDE from discovering blood groups and suggesting their importance in determining success or failure in transfusions, Landsteiner also discovered the pathogenesis of paroxysmal hemoglobinuria, the viral nature of poliomyelitis, the use of alcoholic extracts in the Wassermann tests, that remarkable work which demonstrated the chemical basis of serologic specificity, cultivation of Rickettsia in tissue culture, the theory of hypersensitiveness to drugs and methods for affecting sensitization.

Landsteiner informed me many years ago of how he "put one over" on Ehrlich. The latter had interpreted the discoloration in the finger, i.e., hemolysis in the finger tourniquet test in paroxysmal hemoglobinuria, as due to a vital factor in the endothelium of the blood vessels. With much glee and a merry twinkle in his eye, Landsteiner told me how he with Donath demolished Ehrlich's vital factor when they demonstrated hemolysis in the test tube containing patient's serum and red cells previously chilled and then incubated at body temperature to allow for the action of complement.

The viral nature of polio virus was almost missed because all his rhesus monkeys inoculated with emulsion of spinal cord from an affected patient had severe diarrhea. But out of the corner of his eye, as he was about to leave the animal room, he noticed that one of the monkeys was limping. This led to the successful transfer of the virus to other monkeys.

Socially he was very shy but was most at home with younger co-workers over a schooner of beer. His style of writing was terse and compact. He loved the long, meaningful sentences so characteristic of German writers. It did not take him long to master English, but when angry he reverted to his native German with some "divine" morsels.

My Introduction to Karl Landsteiner

A. S. WIENER, M.D.

MY VERY first meeting with Karl Landsteiner was the direct result of a blunder which I had made in my very first paper, published in 1929. After this inauspicious start, our acquaintance developed into a close association and friendship that continued uninterruptedly until his death in 1943.

As a sophomore medical student, under the guidance of Dr. Silik H. Polayes, I carried out some studies on the blood groups, little suspecting that this was the beginning of my life work. My first paper was a theoreticostatistical analysis of the three theories of heredity of the A-B-O groups, which were being debated at that time, namely, multiple alleles, independent assortment, and linkage, and I concluded that the evidence supported Bernstein's multiple allele theory. Shortly after this was published, I received a telephone call from Philip Levine that Karl Landsteiner with

whom he was working at the Rockefeller Institute (they had recently announced their discovery of the M-N types) wished to see me. As a stripling medical student I approached this interview with awe and trepidation.

It turned out that with the gullibility that comes with inexperience, I had quoted uncritically the assertion of a Japanese worker that isoagglutination was in vogue in China and Japan as early as the 12th century. When I met Dr. Landsteiner I found that despite his impressive and stern demeanor, he was a kindly and helpful scientist who instead of castigating me for my blunder gently suggested that I investigate the source of the claim. A volume was obtained from the Army Medical Library in Washington, containing ancient Chinese and Japanese folklore. According to this source, any person claiming to be an heir to an estate was subjected to the following test: His finger was pricked and a drop of his blood permitted to drip onto the dead person's skeleton; if the blood soaked in he was an heir, otherwise he was an impostor. In a second test to determine whether two persons were related, drops of their blood were allowed to fall into a basin of water—if the drops came together they were indeed related; if the drops flowed apart, they were not.

This early experience taught me not only the obvious lesson to consult all original sources myself rather than to depend on second hand information, but also to be more critical of all claims of highly improbable observations. It is one of the earmarks of the credulous and unreliable investigator that in his very first attempt he claims to have made some fantastic discovery, or describes a finding that is highly improbable *a priori*. As examples may be cited a report in a leading blood journal claiming to be able to determine the A-B-O groups and subgroups of A from the shape of plasma clots, and the spate of papers in recent blood group literature on supposed associations between particular A-B-O groups and diseases such as peptic ulcer, fractured femur and the like.

To avoid repetition of my early blunder, I consulted Dr. Landsteiner regularly from 1929 until his death in 1943, about any results I was planning to publish, and he gave unstintingly of his time and help. Whatever excellence my early book on Blood Groups and Transfusion may have is largely due to his painstaking efforts in checking the manuscript. Indeed, it was this close contact with Karl Landsteiner that led finally to our collaborative studies, the acme of which was our discovery of the Rh factor.

Editorial Note

It is appropriate to dedicate the first issue of TRANSFUSION *to the man who laid the foundations for this field of science. 1961 is the diamond jubilee of the publication of the description of "the so-called Landsteiner reaction" in the Wiener Klinische Wochenschrift (**14: 1132, 1901**). The translation which follows was prepared through the courtesy of Professor A. L. Kappus, Chairman of the Department of Microbiology at the Marquette University School of Medicine. Dr. Kappus made every effort to preserve the flavor of the original article which was made difficult by the involved construction of the academic German in use at the turn of the century. Those who are interested may determine Dr. Landsteiner's blood group by studying his tables. This observation serves to underline the importance of the procedure of "proving the blood groups" by the method introduced by Karl Landsteiner.*

On Agglutination of Normal Human Blood

DR. KARL LANDSTEINER

Assistant at the Pathological-Anatomical Institute, Vienna

SOME time ago I observed and reported that serum of normal humans frequently agglutinates red blood cells of other healthy individuals.[1] At that time I was under the impression that this ability of the serum to agglutinate foreign red cells was especially pronounced in some diseases and I believed that this agglutinating ability was related to the strong lytic ability of pathologic sera on normal red cells which was observed by Maragliano[2] many years ago. This concept seemed to be supported by the fact that agglutinating and lytic abilities frequently although not always changed in a parallel fashion. However, the fact that the addition of sodium chloride up to normal concentrations will (although heating will not) destroy this lytic ability of the sera indicates the reactions of Maragliano are not identical to the hemolytic reactions of the blood sera which are being investigated so intensively. Maragliano himself differentiates his observations from the phenomenon of Landois—hemolysis caused by foreign serum—since in Maragliano's case hemoglobin is not only dissolved but also destroyed. A fundamental difference between my observations and those of Maragliano is that in his case the serum acts also upon the red blood cells of the same individual and that his reaction occurs with pathologic blood only. My observations, however, reveal characteristic differences between blood serum and red blood cells of various apparently healthy persons.

The report and pictures presented by Shattock[3] describe a related phenomenon, although he found the reaction only in febrile diseases and failed to see it with normal blood. Shattock thinks that the reaction is connected with the increased coagulability and rouleaux formation of the febrile blood.

The agglutination of human blood by human serum ought to be called isoagglutination according to the nomenclature of Ehrlich and Morgenroth. Shortly after my publication these two investigators reported

TABLE 1. *Concerning the Blood of Six Apparently Healthy Men*

Sera						
Dr. St.	−	+	+	+	+	−
Dr. Plecn.	−	−	+	+	−	−
Dr. Sturl.	−	+	−	−	+	−
Dr. Erdh.	−	+	−	−	+	−
Zar.	−	−	+	+	−	−
Landst.	−	+	+	+	+	−
Rbc's of:	Dr. St.	Dr. Plecn.	Dr. Sturl.	Dr. Erdh.	Zar.	Landst.

experiments in which they injected blood of the same species and thereby succeeded in preparing isoagglutinins and isolysins, that is, sera which act upon the red blood cells of the same species. These very comprehensive experiments confirm the unexpected existence of clearly demonstrable differences between the bloods within one animal species.

In the paper of Ehrlich and Morgenroth[4] the phenomena of isolysis are discussed in regard to the theoretical concepts presented by Ehrlich.

Since the publication of the reports of Shattock and myself a number of investigators studied isoagglutination in man. The fact that the reaction occurs in the blood of healthy individuals renders those papers valueless which consider the reaction specific for certain diseases.[5] Other papers even report observations concerning the intensity and frequency of the reaction in diseases.

Donath[6] found the reaction more frequently in patients with different forms of anemia than in healthy people, though this was not always the case. Ascoli[7] observed the reaction in healthy individuals but noted greater intensity in diseased ones. Eisenberg investigated healthy and diseased persons. He, like others, came to the conclusion that the reaction occurs frequently in diseased but only exceptionally in healthy persons. This, however, contradicts my statements.[8]

As I have given only a very brief report in my paper mentioned above I would like to present the results of some recent experiments. The tables need no interpretation. Equal amounts of serum and of 5 per cent red blood cell suspension in 0.6 per cent sodium chloride were mixed and observed in a hanging drop preparation or in the test tube. (The + sign signifies agglutination.)

A fourth similar table concerning the sera of Table 2 mixed with the red blood cells of Table 1 and some other tested sera, for instance those of one case of hemophilia and one case of purpura, presents entirely identical regularities and is therefore omitted. The investigation of another ten normal persons (in 42 combinations) gave similar results.

These experiments prove that I do not

TABLE 2. *Concerning the Blood of Six Apparently Healthy Puerperal Women*

Sera						
Seil.	−	−	+	−	−	+
Linsm.	+	−	+	+	+	+
Lust.	+	−	−	+	+	−
Mittelb.	−	−	+	−	−	+
Tomsch.	−	−	+	−	−	+
Graupn.	+	−	−	+	+	−
Rbc's of:	Seil.	Linsm.	Lust.	Mittelb.	Tomsch.	Graupn.

TABLE 3. *Concerning the Blood of Five Puerperal and Six Placentae (Cord Blood)*

Sera						
Lust.	+	+	−	−	−	+
Tomsch.	−	−	+	−	−	−
Mittelb.	−	−	+	−	−	−
Seil.	−	−	+	−	−	−
Linsm.	+	+	+	−	−	+
Rbc's of:	Trautm.	Linsm.	Seil.	Freib.	Graupn.	Mittelb.

have to correct my statement. The reaction was given by all of the 22 tested sera from healthy adults. Obviously, this result would not have been obtained if I had not used a number of different red blood cells for the test.

Halban,[9] Ascoli, and finally Eisenberg have already reported on the variable resistance of red blood cells towards the reaction. This is also shown in the above tables. Moreover, there was a peculiar regularity in the reaction of the 22 tested blood specimens. If one disregards some tests with sera from fetal placental blood which do not cause agglutination—Halban also found fetal blood serum rarely agglutinates—the sera may, in most cases, be grouped in three groups.

In a number of cases (Group A) the serum reacts with the red blood cells of another group (B) but not with the red blood cells of Group A; these react with the serum B in an identical way. In the third group C the serum agglutinates the red blood cells of Group A and B but the red blood cells of C are not influenced by the sera of A and B.

Applying the commonly used technical terms it may be said that there exist at least two different types of agglutinins, one in A, another one in B, and both together in C. The red blood cells are inert to the agglutinins which are present in the same serum.

No doubt a report concerning the presence of a few different agglutinins in the cases tested sounds peculiar, although the experiments of Ehrlich and Morgenroth with isolysins showed quite similar conditions; it might be more satisfactory to find another interpretation by further observation.

It is suggested that attention be paid to these regularities in pathological cases.

Eisenberg thinks that the production of agglutinins is due to the resorption of components of red blood cells. This idea is not new; Halban and Ascoli have already presented it as a possible explanation. I did not formerly refer to it because I did not succeed in producing autoagglutination by injecting animals with their own lysed red blood cells.

I believe that Ehrlich does not report any positive results in that line either; Ascoli on the other hand obtained positive but not constant results. The difficulties of the given interpretation are mentioned by Halban. Perhaps the origin of naturally occurring hemagglutinins and normal agglutinins reacting with bacteria require different explanations.

Furthermore, my experiments show that various sera do not act identically as far as agglutination is concerned. Thus, if one believes that their agglutinating ability is developed in response to some autoimmunization due to resorption of cell components one has also to assume individual differences in order to interpret different sera. Actually, there are differences in different red blood cells and even already in fetal blood. Even if one acknowledges the differences of the sera or the red blood cells, the agglutination within one species is just as easy or as difficult to understand as the

agglutination by a serum from another species.

Nonetheless the presented interpretation cannot be disregarded; it is even the most adequate one if the untested experiments of Ascoli are correct. Then the physiological disintegration of the cells of body tissues might well be responsible for the formation of active serum components.

The experiments with sera from infants and animals are well suited to exclude the influence of pathological processes which may have occurred in the past. Halban's experiments also provide evidence against such a relation.

The agglutination described can be performed with serum which is dried and promptly dissolved; I succeeded in obtaining it with blood dried on cotton cloth and dissolved after fourteen days. Provided rapid changes in the agglutinating ability do not occur the reaction may be suited to establish the identity, or more correctly the non-identity, of a blood specimen. In a second test, the six sera of Table 1 gave the same reaction as a fresh specimen obtained nine days previously.[10]

Finally, it might be mentioned that the reported observations may assist in the explanation of various consequences of therapeutical blood transfusions.

References

1. Centralblatt für Bacteriologie XXVII. **8**: 361, v. 10. Februar 1900.
2. XI Congress für innere Medicin. Leipzig 1892.
3. Journ. of Pathol. and Bacteriology. Februar 1900.
4. Berliner klinische Wochenschrift, 1900.
5. Literatur siehe bei Eisenberg, Wiener klinische Wochenschrift, 1901, Nr. 42.
6. Wiener klinische Wochenschrift, 1900, Nr. 22.
7. Münchener medicinische Wochenschrift, 1901, S. 1229.
8. Although Eisenberg attacks my report in his paper, and at the same time confirms it in regard to the blood of patients, he mentions my paper in the bibliography but with no word in his text.
9. Wiener klinische Wochenschrift, 1900, Nr. 24.
10. Dr. Richter and I plan to investigate the reliability of this method.

Obverse and reverse of the Landsteiner
Medal issued by the Netherlands Red Cross in 1933.
(Courtesy of A. S. Wiener, M.D.)

The Role of Complement in the Detection of Blood Group Antibodies: Special Reference to the Antiglobulin Test*

MARGARET J. POLLEY, P. L. MOLLISON

From the Medical Research Council's Experimental Haematology Research Unit, Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, W.2.

SOME blood group antibodies, such as anti-Le^a, sensitize red cells to an antiglobulin serum only if fresh serum is used;[11] and in these cases the antiglobulin serum reacts with complement rather than with the antibody.[7]

When stored sera are tested for the presence of complement-binding antibody, using the indirect antiglobulin test, it may be necessary to add complement in order to be able to detect the antibody. The question then arises whether complement (fresh normal serum) should simply be added to the stored serum or whether the cells should first be incubated with the stored, antibody-containing serum and then, as a separate step, with complement. The answer to this question must depend upon many factors, some of which have so far scarcely been examined. For example, little is known about the extent to which complement may promote the adsorption of blood group antibodies on to red cells or tend to prevent their elution after adsorption; and little is known about the extent to which stored sera may develop anti-complementary properties which might oppose the adsorption of complement. In this paper some of these problems are examined.

* Received August 23, 1960; Accepted October 12, 1960.

Methods

Red Cells: Red cells were usually obtained from samples of blood stored with ACD for not more than three weeks; but in tests for hemolysis only fresh red cells were used. In all cases the cells were washed three times in saline before being used.

Sera Containing Blood Group Antibodies: Sera containing the following antibodies were tested: anti-Le^a: five stored samples (donors C.P., F.G., W.B., E.K., and A.L.) and one fresh sample (donor D.D.); anti-Jk^a: one stored sample (donor J.C.) and one fresh sample (donor C.F.); anti-K: two stored samples (donors P.K. and K.L.) and one fresh sample (donor P.K.); and anti-Fy^a: two stored samples, one of which was far more potent than the other (both from donor R.D.). All these sera contained predominantly incomplete, that is non-agglutinating, types of antibody.

All the examples of anti-Le^a and of anti-Jk^a, gave a positive indirect antiglobulin test only when complement as well as anti-

body was adsorbed on to the cells; one of the examples of anti-K (from donor P.K.) behaved in the same way; the example of anti-Fy^a gave positive indirect antiglobulin reactions in the absence of complement, although the presence of complement greatly enhanced the reactions; the second example of anti-K (donor K.L.) appeared not to bind complement and was selected as a control.

In experiments on the behavior of normal incomplete cold antibody[5] many samples of fresh serum, mainly from donors of groups A_1 and A_1B,[4] were used.

Sera Used as a Source of Complement: In most instances fresh serum was obtained from defibrinated blood from donors of suitable ABO groups. When fresh serum was added directly to anti-Le^a serum, a donor of group Le (a-b-) was chosen; in a subsidiary investigation, the effect of adding fresh serum from donors of various Lewis groups was investigated. An experiment was performed to investigate the effect of storage on the complementary activity of serum. Samples were taken from a single donor (M.P.) at intervals over a period of two months and stored at +37 C., +20 C., +4 C., −20 C. and −50 C. At the end of two months fresh serum was taken from the same donor and this and the stored sera were all tested on the same day.

The samples were tested in three ways: first they were used as a source of complement in the antiglobulin test. Le (a+) red cells were incubated for 90 minutes with EDTA-treated serum containing anti-Le^a (EDTA—ethylene diamine tetra-acetic acid —was added to prevent the binding of complement at this stage of the reaction; the exact procedure is described later). The sensitized cells were then washed and incubated at 37 C. for 20 minutes with varying dilutions of the stored serum before being washed again and, finally, tested with an antiglobulin serum. In the second test the red cells were treated in exactly the same way as in the previous test but were finally allowed to react with an immunoconglutinin serum instead of with an antiglobulin serum. In the third test serial dilutions of the stored sera were added to sheep cells sensitized with bovine antibody and after incubation at 37 C. for 20 minutes, the degree of hemolysis was recorded.

Assessment of Anti-Complementary Properties of Stored Sera: During the course of the work many sera which had been stored at −20 C. were found to have developed anti-complementary properties. The degree of this property was investigated in two different ways:

Inhibition of the lysis of sensitized sheep cells by normal fresh serum. The sera to be tested were first heated to 56 C. for 30 minutes; serial dilutions were then prepared and one volume of each dilution was mixed with an equal volume of a one in 20 dilution of fresh serum and left for 15 minutes at room temperature before being incubated with sheep red cells previously sensitized with bovine antibody. A one in 20 dilution of fresh serum was chosen as this gave only partial lysis (about 40 per cent). After 45 minutes' incubation at 37 C., the mixtures were examined for hemolysis.

Blocking of the adsorption of complement on to sensitized red cells. Fy (a+) red cells were sensitized with varying dilutions of a serum containing anti-Fy^a; this example of anti-Fy^a was capable of binding complement but complement-binding at this stage was prevented by using serum treated with EDTA. The red cells sensitized with anti-Fy^a were washed three times and then incubated for one and a half hours at 37 C. with samples of stored serum which had been treated in various ways; the object of this stage of the test was to provide an opportunity for the adsorption of substances which might block the subsequent adsorption of complement. The cells were then washed three times and incubated at 37 C. for 20 minutes with nor-

mal fresh serum. The cells were finally washed three times and tested with an antiglobulin serum.

Antiglobulin Sera: Antiglobulin sera which contained antibodies against "non-gamma" globulin, i.e., the appropriate $beta_1$ globulins[10, 13] as well as against gamma globulins, were used. As already mentioned, the example of anti-Fy^a sensitized red cells to an antiglobulin serum in the absence of complement but gave better reactions when complement as well as antibody was adsorbed on to the cells. In making tests with this serum, one volume of 0.01 per cent gamma globulin was added to the antiglobulin serum in order to detect adsorbed complement only and not antibody.

Antiglobulin Tests: The effects of incubating cells with antibody and complement simultaneously ("one-stage" tests) were compared with the effects of incubation first with antibody and then as a separate stage with complement ("two-stage" tests).

"One-Stage" Tests: Four volumes of the serum containing a blood group antibody and two volumes of fresh serum (as a source of complement) were added to one volume of a 20 per cent suspension of cells and incubated in a water bath at 37 C. for one and a half hours. The cells were then washed three times and a 10 per cent suspension was prepared. One drop was mixed with one drop of a suitable antiglobulin serum on an opal tile and after being rocked gently for five minutes, examined for agglutination. Results were expressed as "scores."[14]

"Two-Stage" Tests: Four volumes of the serum containing the blood group antibody were mixed with one volume of a 20 per cent suspension of cells and incubated in a water bath at 37 C. for one and a half hours. The cells were then washed three times and all saline was discarded before adding two volumes of fresh serum and incubating at 37 C. for a further 20 minutes. The cells were then washed and tested with antiglobulin serum as before.

It will be noted that in the "one-stage" tests one volume of cells was treated with four volumes of antibody-containing serum and two volumes of fresh serum, whereas in the first stage of the "two-stage" test one volume of cells was treated only with four volumes of antibody-containing serum. To discover whether this slight dilution of the antibody-containing serum influenced the results, further tests were made with one volume of cells, four volumes of antibody-containing serum and two volumes of kaolin-absorbed (i.e. complement-free) serum. It was found that the addition of the two volumes of kaolin-absorbed serum was without effect.

Similarly, to discover whether the second stage of incubation of sensitized cells in fresh serum had any obvious effect on the result apart from the adsorption of complement, cells which had been treated with antibody-containing serum and complement simultaneously ("one-stage" test) were washed and then incubated for 20 minutes at 37 C. with two volumes of kaolin-treated serum. It was found that this further period of incubation had no effect on the results.

In performing the "two-stage" tests, antibody-containing serum was treated in various ways to inactive complement; methods employed were:

(a) Heating to 56 C. for 30 minutes.

(b) Addition of EDTA. Preliminary experiments were carried out to discover how much EDTA had to be added to fresh serum to prevent the binding of complement. On the assumption that one molecule of EDTA binds one atom of calcium, the quantity required to chelate calcium is approximately 1.5 mg. of the disodium salt of EDTA for each ml. of serum. Serum containing anti-Le^a was treated with 4 mg./ml. and used to sensitize (Le (a+) red cells. The cells were washed three times and then

TABLE 1. *Results of Using Serum Stored at Temperatures Between —50 C. and +37 C. as a Source of Complement in Three Different Tests*

	Reactions obtained with various dilutions of serum (expressed as reciprocals)																			
	Fresh				Stored 1 day				Stored 1 week				Stored 4 weeks				Stored 2 months			
	1	4	16	64	1	4	16	64	1	4	16	64	1	4	16	64	1	4	16	64
Antiglobulin tests																				
Serum stored at +37 C.					—	(+)	—	—	..	..	..	..	..	..	..	..	..	..	..	..
" " " +20 C.					++	++	(+)	—	..	..	..	..	..	..	..	..	..	..	..	..
" " " + 4 C.	++	++	++	—	+	+++	+	—	(+)	++	(+)		—	—	—	—	—	—	—	—
" " " —20 C.					++	++	++	—	++	++	++	—	++	++	+	—	++	++	(+)	—
" " " —50 C.					++	++	++	—	++	++	++	—	++	++	++	—	++	++	+	—
Tests with immuno-conglutinin																				
Serum stored at +37 C.					—	+	—	—	..	..	..	..	..	..	..	..	..	..	..	..
" " " +20 C.					+	++	++	(+)	..	..	..	..	..	..	..	..	..	..	..	..
" " " + 4 C.	++	++	++	—	+	++	+	—	(+)	++	+	—	—	—	—	—	—	—	—	—
" " " —20 C.					(+)	++	+	—	+	++	+	(+)	(+)	+	+	—	—	(+)	(+)	—
" " " —50 C.					(+)	++	+	—	++	++	+	+	+	++	+	(+)	++	++	+	(+)
Lysis of sensitized sheep cells																				
Serum stored at +37 C.					c	c	++	—	..	..	..	..	..	..	..	..	..	..	..	..
" " " +20 C.					c	c	+++	—	..	..	..	..	..	..	..	..	..	..	..	..
" " " + 4 C.	c	c	c	+	c	c	c	+	c	c	+++	(+)	c	+++	+	—	c	+	—	—
" " " —20 C.					c	c	+++	++	c	c	+++	++	c	c	+++	++	c	c	++	(+)
" " " —50 C.					c	c	c	+	c	c	c	+	c	c	++++	+	c	c	++++	+

c = complete hemolysis
+++, ++, +, (+) = decreasing degrees of hemolysis or agglutination
.. = not tested

inculcation of [illegible] amou[illegible] ing f[illegible] had [illegible]

Ce[illegible] which[illegible] ected[illegible] untre[illegible] with[illegible] leen[illegible] reacti[illegible] adop[illegible] of EI[illegible] cure[illegible] solut[illegible] in su[illegible] 7 and[illegible] for th[illegible]

A s[illegible] 0.3% [illegible] solutio[illegible] and al[illegible] EDTA[illegible] of seru[illegible]

(c) [illegible] ration[illegible] by B[illegible] serum[illegible] incub[illegible] mixtu[illegible] perma[illegible] exper[illegible] would[illegible] activi[illegible] kaoli[illegible] plete[illegible]

Ab[illegible] Sera[illegible] volum[illegible] tant[illegible] were[illegible] const[illegible]

Te[illegible] ("**I-K[illegible]** descri[illegible] a ral[illegible] type;[illegible] in th[illegible]

incubated with fresh serum to which amounts of Na_2H_2 EDTA (see below) varying from 0.25 mg. to 4.0 mg./ml. of serum had been added.

Cells incubated with fresh serum to which 1 mg. per ml. had been added reacted almost as well as cells incubated with untreated fresh serum; but cells incubated with serum to which 2 mg. per ml. had been added gave a completely negative reaction. Accordingly the routine was adopted of adding the equivalent of 4 mg. of EDTA to each ml. of serum; the procedure actually adopted was to make up a solution of K_2H_2 EDTA, adjust the *p*H to 7 and then to place appropriate amounts in suitable containers and dry them ready for the addition of fresh serum.

A solution containing 4.45% K_2H_2 EDTA and 0.3% NaOH was prepared. 0.1 ml. amounts of this solution were pipetted into small glass containers and allowed to dry at 37 C. This provided 4 mg. of EDTA in each container which was used for 1 ml. of serum.

(c) Absorption with kaolin. The preparation used was "kaolin heavy" supplied by British Drug Houses. 1 ml. of fresh serum was added to 0.5 g. of kaolin and incubated at 37 C. for 15 minutes. The mixture was then centrifuged and the supernatant serum recovered. (Preliminary experiments showed that this treatment would remove all detectable complement activity whereas absorption with 0.25 g. kaolin per ml. diminished but did not completely remove complement activity.)

Absorption and Elution of Antibodies: Sera were absorbed with one-tenth of their volume of suitable red cells; the supernatant was retained for testing and the cells were heated to 56 C. for ten minutes, with constant shaking, to elute the antibody.

Tests with an Immuno-Conglutinin ("I-K") Serum: These were carried out as described by Mollison and Polley,[12] using a rabbit serum of the "auto-stimulation" type;[3] only human complement was used in the tests.

Hemolytic Tests: The tests were carried out as part of the two-stage indirect antiglobulin test. Thus at the end of the period of incubation with complement, the degree of lysis was noted and if this was not excessive the cells were washed and tested with antiglobulin serum.

Results

Stored Serum as a Source of Complement (see Table 1). *Serum stored at −50 C.:* In all tests, serum stored at −50 C. for periods up to two months gave results which were almost indistinguishable from those given by fresh serum, although in two cases the undiluted stored serum gave definitely weaker reactions in the immuno-conglutinin tests. This is discussed again later in considering the development of anticomplementary properties on storage.

Sera stored at −20 C: Progressive deterioration was most evident in the tests with an I-K serum; as in the samples stored at −50 C., the undiluted serum sometimes gave weaker reactions than the serum diluted one in four. Serum stored for two months gave only slightly weaker reactions than fresh serum when used as a source of complement in the antiglobulin test or in the lysis of sensitized sheep cells.

Samples of the same batch of serum were also stored at −20 C. for six months and tested at monthly intervals. There was progressive deterioriation so that after six months the sera were only weakly active as a source of complement in the I-K test. However, in the antiglobulin test and in the lysis of sensitized sheep cells, serum stored for six months was still about 75 per cent as active as fresh serum, as judged by the maximum dilution of serum giving positive results.

Sera stored at +4 C.: Sera stored for one week were definitely less satisfactory than fresh serum in the antiglobulin test and serum stored for four weeks was completely inactive. In the I-K tests deterioration at one week was less evident but serum

TABLE 2. *Anti-Complementary Activity of Serum Stored at −20 C.*

Donor of stored serum	Period of storage	Lysis of sensitized sheep cells in mixtures containing following dilutions* of stored serum (as reciprocals)					
		1	2	4	8	16	32
Control**	Nil	++	++	++	++	++	++
M.P.	6 weeks	(+)	+	+	+	++	++
"	6 months	−	(+)	+	+	++	++
A.L.	2 years	(+)	+	++	++	++	++
A.S	3 years	(+)	+	+	++	++	++
W.B.	5 years	−	−	−	W	(+)	+
" absorbed with kaolin	"	++	++	++	++	++	++

* 1 volume of a one in 20 dilution of fresh serum in saline was added to an equal volume of each dilution of heated stored serum and incubated at room temperature for 15 minutes before the addition of sensitized sheep cells.

** Heated fresh serum was used as a control.

++ = Maximum lysis (no inhibition).

+, (+), − = Decreasing degrees of lysis (increasing degrees of inhibition).

stored for four weeks gave completely negative results. As a source of complement in the lysis of sensitized sheep cells serum stored for one week was only slightly less satisfactory than fresh serum; serum stored for two months was definitely less satisfactory but was still active.

Sera stored at +20 C. and at +37 C.: Complement activity was tested after only one day's storage at +20 C. and +37 C. After one day's storage at +20 C. there was evidence of slight deterioration; after storage at 37 C. the serum was almost inactive as a source of complement in tests with an antiglobulin serum or I-K serum but still produced considerable lysis of sensitized sheep cells.

Anti-Complementary Properties of Stored Serum: Many samples of serum which had been stored at −20 C. for periods varying from a few weeks to a few years were tested, as described in "Methods." Table 2 shows the effects on the inhibition of the lysis of sensitized sheep cells by normal fresh serum. All sera tested were anti-complementary to some extent and on the whole there was a tendency for the sera to become increasingly anti-complementary as the period of storage increased. After absorption with kaolin the stored sera were no longer anti-complementary.

Fresh serum heated to 56 C. for 30 minutes failed to inhibit an equal volume of a one in 20 dilution of fresh (unheated) normal serum, using as a control saline added to a one in 20 dilution of fresh serum.

A few experiments carried out with sera stored at +4 C. showed that the anti-complementary property developed more rapidly at this temperature; see also Table 1 in which, using serum stored for one week, the reactions with undiluted serum are definitely weaker than those of diluted serum.

Table 3 demonstrates the anti-complementary properties of sera in another way. As described in "Methods," Fy^a-sensitized red cells were incubated with stored serum, then with fresh serum to see if the preliminary incubation with stored serum prevented the subsequent adsorption of normal complement. As Table 3 shows, treatment of sensitized cells with untreated stored serum diminished their ability to take up complement on subsequent expo-

sure to fresh serum; heated stored serum had less "blocking" effect and EDTA-treated or kaolin-absorbed stored serum did not exert any "blocking" effect at all. Treatment of the sensitized cells with fresh serum or with heated fresh serum did not interfere with their ability to take up complement subsequently.

One-Stage and Two-Stage Antiglobulin Tests

Results with Fresh Antibody-Containing Sera: As Table 4 shows, results with anti-Le^a were virtually the same in all tests; for example, incubation with EDTA-treated antibody-containing serum followed by separate incubation with complement gave reactions which were just as strong as those obtained by simple incubation with the untreated serum.

With anti-K and anti-Jk^a the results were very different. Incubation with treated antibody-containing serum and complement simultaneously gave far better results than incubation with the treated antibody and complement in two successive stages. In the one-stage test, kaolin-absorbed anti-K gave good reactions whereas kaolin-absorbed anti-Jk^a gave very weak reactions, suggesting that kaolin actually adsorbed the antibody.

Results with Stored Antibody-Containing Sera: The results with stored sera were very different from those obtained with fresh sera. With fresh antibody-containing sera, one-stage tests with untreated sera invariably gave as strong reactions as those obtained by any other method. On the other hand, with stored sera better results were usually obtained by some other method. Thus, with anti-Le^a, the best results were obtained in two-stage tests, particularly when the cells were first incubated with kaolin-treated or EDTA-treated anti-Le^a in the first stage. With anti-K (donor P.K.) the best results were obtained in a one-stage test in which the cells were incubated with kaolin-treated anti-K and with normal fresh serum. With anti-Fy^a the best results were obtained in a two-stage test using EDTA-treated anti-Fy^a.

With stored anti-Jk^a serum, easily the best results were obtained in the one-stage

TABLE 3. *Effect of Stored Serum in Interfering with the Adsorption of Complement*

Serum used in second stage of test (See legend below table)	Reactions of cells sensitized with following dilutions of anti-Fy^a (as reciprocals)					
	2	4	8	16	32	64
Stored serum						
Untreated	++	++	+	+	(+)	−
Heated to 56 C. for 30 minutes	+++	+++	++	++	+	(+)
Kaolin-treated	+++	+++	+++	++	++	+
EDTA-treated	+++	+++	+++	++	++	+
Fresh serum						
Untreated	+++	+++	+++	++	++	+
Heated to 56 C. for 30 minutes	+++	+++	+++	++	++	+
Kaolin-treated	+++	+++	+++	++	++	+
EDTA-treated	+++	+++	+++	++	++	+

As a first stage, Fy$^{(a+)}$ red cells were sensitized with varying dilutions of EDTA-treated anti-Fy^a; they were then (second stage) incubated either with stored serum treated in various ways or, as a control, with fresh serum similarly treated; finally (third stage) they were incubated with fresh normal serum before being tested with an antiglobulin serum.

TABLE 4. *Antiglobulin Tests with Various Antibody-Containing Sera, Fresh and Stored*

Treatment of antibody-containing serum	Results of indirect antiglobulin tests as "scores"						
	Fresh sera			Stored sera			
	Anti-Le^a	Anti-K	Anti-Jk^a	Anti-Le^a	Anti-K	Anti-Fy^a	Anti-Jk^a
	(D.D.)	(P.K.)	(C.F.)	(W.B.)	(P.K.)	(R.D.)	(J.C.)
One-stage tests							
Untreated	47	34	9	22	28	43	23
Heated (56 C. 30 min.)	43	26	3	13	27	41	11
Absorbed with kaolin	47	34	2	38	38	10	3
Two-stage tests							
Untreated	47	27	9	29	25	43	15
Heated (56 C. 30 min.)	44	14	0	24	24	45	7
Absorbed with kaolin	49	17	0	46	24	25	0
EDTA-treated	47	17	0	46	25	56	11

In one-stage tests, cells were incubated with a mixture of antibody-containing serum and fresh serum (as a source of complement). In two-stage tests, cells were incubated first with antibody-containing serum, then, after washing, were incubated with fresh serum as a source of complement. In both one and two-stage tests serial dilutions of the antibody-containing serum were tested and the results expressed as a total score.

When fresh antibody-containing sera were used "untreated," the results in the one-stage and two-stage tests were expected to be identical since in both tests the cells were first incubated with antibody and complement simultaneously.

test with untreated serum; two-stage tests gave definitely inferior results.

Effect of Lewis Group of Serum Used as a Source of Complement in One-Stage and Two-Stage Tests: As Table 5 shows, in one-stage tests (i.e. incubation with antibody and complement simultaneously) the best results were obtained using Le (a–b–) serum as a source of complement and when Le (a+b–) serum was used the reactions were completely inhibited. In two-stage tests (incubation with antibody, then after washing the cells, incubation with complement) equally good results were obtained with Le (a+) and Le (a–) sera.

One and Two-Stage Tests for Hemolysis: All five stored anti-Le^a sera were tested. With four of these, no hemolysis could be

TABLE 5. *Effect of Lewis Group of Sera Used as a Source of Complement in "One-Stage" and "Two-Stage" Antiglobulin Tests With Cells Sensitized With Stored Anti-Le^a Serum*

Test	Fresh serum used as a source of complement	Dilutions of anti-Le^a (reciprocals)					
		2	4	8	16	32	64
"One-stage"	Le (a+b–) serum	–	–	–	–	–	–
	Le (a–b+) "	+	(+)	–	–	–	–
	Le (a–b–) "	++	++	+	–	–	–
"Two-stage'	Le (a+b–) "	+++	+++	++	+	(+)	–
	Le (a–b+) "	+++	+++	++	+	(+)	–
	Le (a–b–) "	+++	+++	++	+	(+)	–

In "one-stage" tests Le (a+) cells were incubated with anti-Le^a and fresh serum simultaneously; in "two-stage" tests Le (a+)' cells were incubated first with anti-Le^a and then washed and incubated with fresh serum.

elicited with the untreated serum either when complement was added simultaneously or when the cells were first treated with antibody and then separately with complement, but if the cells were first incubated with EDTA-treated anti-Le^a serum, then washed and treated with complement, hemolysis occurred in every case. Accordingly all five sera were treated with EDTA and re-tested. Le (a+) cells were incubated with EDTA-treated serum, then washed and treated with fresh serum. In all cases there was hemolysis, although the degree varied (see Table 6). The unhemolysed cells were washed and tested with antiglobulin serum and, as the figure shows, the score obtained was approximately proportional to the degree of hemolysis.

TABLE 6. *Comparison of Hemolytic Test and Indirect Antiglobulin Test With Five Anti-Le^a Sera*

Donor of anti-Le^a serum (initials)	Hemolytic test (score)	Antiglobulin test (score)
C.P.	3	25
F.G.	5	25
W.B.	10	35
E.K.	15	46
A.L.	27	56

Cells were first incubated with EDTA-treated anti-Le^a serum, then washed and incubated with fresh normal serum.

After scoring the degree of hemolysis, the unhemolyzed cells were washed and tested with an antiglobulin serum.

Further Tests on the Effects of Heating Sera: As Table 4 shows, heated, fresh, antibody-containing sera gave weaker reactions than untreated fresh sera in one-stage tests, suggesting either that the antibody was damaged by heating or that despite some results referred to above, heating made the sera anti-complementary. To investigate this further, sera were heated to 56 C. for varying periods and tested before and after absorption with kaolin, using a one-stage test. As Table 7 shows, treatment of the heated serum with kaolin improved the reactions. Thus whereas sera heated to

TABLE 7. *The Effect on Anti-Le^a and Anti-K Sera of Heating to 56 C. for Varying Periods*

	Period of heating at 56 C.	Scores (indirect antiglobulin test)		
		Anti-Le^a	Anti-K (complement-binding)*	Anti-K (not complement-binding)**
Antibody-containing serum (untreated)	Nil	38	17	13
	30 minutes	34	13	13
	60 "	30	11	11
	90 "	25	8	11
	120 "	25	..	3
Antibody-containing serum (absorbed with kaolin)	Nil	38	17	13
	30 minutes	38	17	13
	60 "	32	16	13
	90 "	30	14	11
	120 "	28	..	3

Samples of antibody-containing sera were heated for various periods and each sample was then divided into two parts, one of which was absorbed with kaolin. Fresh normal serum was then added to the anti-Le^a serum and the complement-binding anti-K serum and all sera were then tested using the indirect antiglobulin test.

* Donor: P.K.

** Donor: K.L.

TABLE 8. *Effect of Heating Stored Anti-Le^a Sera on Results Obtained in Various "Two-Stage" Tests*

Donor of anti-Le^a serum (initials)	Treatment of anti-Le^a serum	Hemolysis	Antiglobulin test	Test with immuno-conglutinin
F.G.	Unheated	+	W	−
	Heated	+++	+++	+++
E.K.	Unheated	+	−	−
	Heated	++++	++++	+++
A.L.	Unheated	++	W	−
	Heated	+++	+++	+++

Each sample of anti-Le^a serum was divided into two aliquots, one of which was heated to 56 C. for 30 minutes. Both aliquots were incubated with Le(a+) cells which were then washed and incubated with fresh normal serum. After scoring the degree of hemolysis, the unhemolyzed cells were tested firstly with an antiglobulin serum and secondly with an I-K serum. The results shown refer to the reactions of undiluted anti-Le^a serum.

56 C. for 30 minutes gave weaker reactions than unheated serum, sera heated to 56 C. for 30 minutes and then absorbed with kaolin gave reactions as strong as those of unheated serum. Serum heated to 56 C. for periods longer than 30 minutes gave weaker reactions than unheated serum even after absorption with kaolin.

The fact that heating to 56 C. for more than 30 minutes damaged the antibody was confirmed by tests with the example of anti-K (donor K.L.) which did not bind complement at all. As seen in Table 7, there was a progressive fall in titre when the period of heating to 56 C. exceeded 30 minutes.

Although fresh or stored sera heated to 56 C. for only 30 minutes gave weaker reactions than unheated sera in one-stage tests, in two-stage tests heated stored sera usually gave better reactions than unheated stored sera. Thus, out of four stored anti-Le^a sera tested, three gave very much better reactions in two-stage tests if they were first heated to 56 C. for 30 minutes (Table 8). The serum of one donor reacted as well in two-stage tests when it was not first heated (donor W.B.—Table 4). These findings are discussed later.

Effect of Complement on Absorption and Elution of Antibodies: Table 9 shows the results of absorbing antibody-containing

TABLE 9. *Results of Absorbing Sera With and Without the Presence of Complement*

	Results of antiglobulin test (scores)				
	Fresh anti-Le^a (D.D.)	Fresh anti-K (P.K.)	Fresh anti-Jk^a (C.F.)	Stored anti-Fy^a (R.D.)	
				Sample 1	Sample 2
Untreated serum*	47	27	16	89	44
" " after absorption	3	2	3	89	7
Eluate from cells	20	11	8	8	0
"Inactivated" serum**	47	17	6	94	47
" " after absorption	3	12	5	105	3
Eluate from cells	40	9	0	23	0

* An equal volume of fresh normal serum was added to stored anti-Fy^a serum.

** Anti-Le^a and anti-K sera were treated with kaolin; anti-Jk^a and anti-Fy^a sera were heated to 56 C. for 30 minutes. An equal volume of kaolin-absorbed fresh serum was added to the heated anti-Fy^a serum.

The sera were absorbed with 1/10 of their volume of packed red cells.

ing sera in the presence or absence of complement.

Complement was removed from anti-Le^a and anti-K sera by absorption with kaolin but the method could not be used with anti-Fy^a or with anti-Jk^a since kaolin absorbed these antibodies. These sera were therefore inactivated by heating to 56 C. for 30 minutes.

The anti-Le^a anti-Jk^a and anti-K used in these tests were all fresh sera. Only stored anti-Fy^a serum was available but two samples taken from the donor at different times were tested; these were first heated to inactivate any residual complement and then mixed with an equal volume of fresh normal serum.

Table 9 shows that anti-Jk^a and anti-K were much better absorbed when complement was present but anti-Le^a and anti-Fy^a were as well absorbed when complement was absent. Only the second sample of anti-Fy^a illustrates this point, the first sample being so potent that its titre was not affected by absorption with 1/10th volume of cells.

Eluates: Considerably more anti-Le^a was found in the eluate made from cells sensitized with kaolin-treated or EDTA-treated anti-Le^a serum (Table 9) than from cells treated with antibody and complement, suggesting that complement interferes with the release of the antibody.

With anti-Jk^a and anti-K the comparison of eluates was complicated by the fact that, as shown above, more of these two antibodies was absorbed in the presence of complement. For example, the finding that no anti-Jk^a could be eluted from cells treated with heated anti-Jk^a (no complement added) and that some anti-Jk^a could be eluted from cells treated with fresh anti-Jk^a serum could be at least partly explained by the fact that less anti-Jk^a was absorbed from the heated serum.

Relatively little antibody was eluted from Fy^a-sensitized cells and then only from the cells sensitized with the more potent sample of the serum. As with the sample of anti-Le^a, definitely more antibody was eluted from cells which had adsorbed antibody alone than from those which had adsorbed antibody and complement.

TABLE 10. *Experiments With Normal Incomplete Cold Antibody*

Human group O red cells treated at 0 C. with:	Reactions with Immuno-Conglutinin Serum
Fresh AB serum	++++
Heated AB serum	−
Heated AB serum + fresh horse serum	+++
(Heated AB serum absorbed with group O cells) + fresh horse serum	+++
(Fresh AB serum absorbed with group O cells) + fresh horse serum	+

Experiments with Normal Incomplete Cold Antibody: One sample of AB serum, unlike all the other samples of AB or A sera tested, appeared at first to contain virtually no normal incomplete cold antibody, that is to say, when 20 volumes of fresh AB serum was mixed with one volume of group O cells and left at 0 C. for one hour and the red cells were then washed and tested with an antiglobulin serum, only a very faint positive reaction was observed. It was suspected that the AB serum might be anticomplementary and this was investigated by testing its ability to inhibit the lysis of sensitized sheep cells by fresh normal human serum. The AB serum was found to be definitely inhibitory; thus one volume of a one in eight dilution of heated AB serum completely inhibited the ability of an equal volume of a one in 20 dilution of fresh human serum to bring about the lysis of sensitized sheep cells.

Further experiments were performed to find out whether the normal incomplete cold antibody was damaged by heating to 56 C. for 30 minutes and if horse complement would serve instead of human complement to bind the antibody on to human red cells.

As expected, when group O cells were mixed with 20 volumes of previously heated AB serum (56 C. for 30 minutes) and left at 0 C. for one hour, they were not agglutinated by an immuno-conglutinin serum. If the cells were now washed and treated at 37 C. with fresh human serum, again they would not react with an I-K serum; but if group O cells were mixed with heated human AB serum and fresh horse serum and the mixture was left at 0 C. for one hour, the cells were then agglutinated by an I-K serum (Table 10).

In an attempt to confirm that the incomplete cold antibody was adsorbed on to cells only in the presence of complement, fresh and heated AB serum were absorbed with group O cells; fresh horse serum was then added to the absorbed sera which were now mixed with a new aliquot of group O cells and left at 0 C. for one hour; the cells were then tested with I-K serum. As Table 10 shows, incomplete cold antibody appears to have been absorbed when cells were exposed to fresh AB serum but not when they were exposed to heated AB serum.

Discussion

The tests with sera stored at various temperatures show not only the deterioration of complement, which was expected, but also show the regular development of anti-complementary properties. The regular development of anti-complementary properties on storage of normal sera at room temperature has previously been described[1] but no references have been found to the regular development of these properties in sera stored in the cold. These properties seem to be of two kinds: first the development of a substance which may be bound to sensitized red cells and then prevent the adsorption of normal complement; second, the ability, when mixed with fresh serum, to diminish its complementary activity.

The conception of a partly-damaged complement—"complementoid"—which can combine with sensitized cells and prevent the action of normal complement is to be found in many papers published between about 1900 and 1910; see for example Ehrlich and Sachs[8] and Bordet[2] interpreting the work of Gay[9]. Many observations in this paper appear to support this conception; for example the finding that stored sera treated with EDTA or absorbed with kaolin frequently bring about the binding of more complement in two-stage tests than untreated stored sera. The most direct evidence in support of the presence of "complementoid" in stored serum is provided by the observations recorded in Table 3. Thus if cells sensitized with anti-Fy^a are exposed to stored serum, their ability to take up normal complement subsequently is diminished.

Heating to 56 C. for 30 minutes apparently reduces the ability of "complementoid" to bind on to sensitized cells because in two-stage tests heated sera may give stronger reactions than unheated serum (Table 8).

The effects of heating to 56 C. are complex for, apart from damaging "complementoid" (and complement), antibody itself is damaged. The damage to antibody appears to be negligible when the period of heating is limited to 30 minutes.

One further effect of heating to 56 C. seems to be the production of a substance, possibly heat-denatured protein, which is capable of binding or inactivating complement when the heated serum is mixed with fresh serum. This would explain the fact that when serum containing a complement-binding antibody is heated to 56 C. for 30 minutes and then mixed with fresh serum, the reactions obtained are weaker than those of unheated fresh serum, whereas heated serum absorbed with kaolin reacts as strongly as unheated serum. Nevertheless it is puzzling that fresh serum heated to 56 C. for 30 minutes does not appear to inhibit the lysis brought about by diluted fresh human serum added to sensitized sheep cells.

TABLE 11. *Some Ideas About Anti-Complementary Effects*

		Effects of	
		Kaolin	EDTA
Storage → $\overline{C'}$	Competes with C′	Adsorbs $\overline{C'}$ (and C′)	Blocks binding of $\overline{C}$ (and of C′)
Heating → $\overline{\delta}$	Deviates C′ (and $\overline{C'}$)	Adsorbs $\overline{\delta}$	? No effect

C′ = Complement $\overline{C'}$ = Complementoid $\overline{\delta}$ = Denatured gamma globulin

It is possible that some stored sera already contain denatured protein which itself adsorbs complement and complementoid. This would explain why occasional samples of stored serum containing complement-binding antibody do not give better reactions in two-stage tests after being heated, i.e. complement may already have been bound by denatured protein (see serum of W.B.—Table 4). Ideas about the behavior of complementoid and denatured globulin are summarized in Table 11.

If complement played no part in promoting the adsorption or binding of antibodies to antigens, two-stage tests using EDTA-treated antibody-containing serum would be desirable as a routine so as to overcome any anti-complementary activity in the antibody-containing serum. However there is good evidence that in some instances complement does play a part in fixing the antibody to the antigen. In the case of the normal incomplete cold antibody[5] it appears that complement is essential for the binding since the antibody is not absorbed from sera in the absence of complement (see Table 10). One other cold antibody occurring in human sera is known which is bound better in the cold when complement is present, namely the Donath-Landsteiner antibody (see Dacie[6]).

Of the blood group antibodies investigated in the present paper, anti-K and anti-Jk^a seem to bind much better in the presence of complement. The binding of anti-Le^a and anti-Fy^a does not seem to be affected although anti-Le^a can be eluted far more easily from cells which have adsorbed antibody alone, that is without complement, as Table 9 shows and as Stratton[15] has also found.

When it is suspected that an antibody is present and an ordinary indirect antiglobulin test fails to reveal it, it is worth trying some of the modified tests discussed in this paper, for example, incubation with EDTA-treated or kaolin-treated antibody-containing serum, followed by incubation with complement. In other cases, simple incubation of the untreated serum with added fresh serum in a one-stage test may give the best results.

Although anti-complementary effects are found much more commonly in stored sera than in fresh sera, it should be remembered that even fresh samples of serum from normal donors may occasionally be anti-complementary and that this may mask the presence of a complement-binding antibody as shown by the example reported here of a sample of fresh AB serum which at first seemed not to contain the normal incomplete cold antibody.

Summary

1. Sera stored at −20 C. regularly become anti-complementary. This is due apparently to the development of "complementoid," a form of complement which may be adsorbed on to sensitized red cells and block the adsorption of normal complement.

2. The adsorption of "complementoid" can be prevented by first treating the antibody-containing serum with EDTA; fresh complement can then be added as a separate stage.

3. Two-stage tests are suitable only for the detection of certain complement-binding antibodies, e.g. anti-Le^a and anti-Fy^a which are adsorbed just as well in the absence of complement as in its presence. Anti-K and anti-Jk^a are better adsorbed when complement is present and therefore a one-stage test should be used for their detection. If sera containing the antibody are anti-complementary, excess fresh serum can be added to provide sufficient complement.

4. Heating sera to 56 C. for only 30 minutes produces negligible damage to blood group antibodies but causes the development of an anti-complementary property. Thus when the heated serum is added to fresh normal serum, the complement activity of the fresh normal serum is diminished, at least in certain tests. This property of the heated serum can be removed by absorption with kaolin. Kaolin also absorbs "complementoid" and complement but is not suitable for the routine treatment of all anti-complementary sera since it also partially absorbs some blood group antibodies (e.g. anti-Jk^a).

5. Some complement-binding blood group antibodies, e.g. anti-Le^a, are much more easily eluted from cells which have adsorbed antibody alone without complement.

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Changes in the Recipient's Plasma Hemoglobin Concentration After Transfusion with Stored Blood *

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IT IS generally accepted that the *in vivo* destruction of compatible nonviable stored red cells is not accompanied by grossly visible hemoglobinemia and presumably is accomplished by some sort of *extravascular* mechanism.[13] However, in the precise study of hemolytic phenomena in patients who may require blood transfusion, it would be useful to know whether the transfusion of stored blood has any chemically detectable effect on the recipient's plasma hemoglobin concentration. This would apply to studies on patients with primary or secondary hemolytic anemias; to studies on the possible hemolytic effects of anesthesia, of drugs or of surgery; and to the investigation of suspected hemolytic transfusion reactions.

Two recent technical developments have made possible the more accurate study of subtle hemolytic phenomena: (1) definition of a method for blood sampling which minimizes artifactual hemolysis incident to blood collection and (2) a further modification of the benzidine method so as to provide a fourteen-fold increase in its sensitivity for hemoglobin measurement.[9] Accordingly, these two methods have been applied to study changes in the recipient's plasma hemoglobin concentration following transfusion. The present report illustrates the sensitivity of the above described laboratory methods for detecting the intravascular destruction of very small volumes of transfused red cells and presents the results of studies on the recipients of transfusions of blood stored up to 42 days under conventional blood bank conditions.

Methods

I. Special Studies on the Destruction of Small Volumes of ABO-Incompatible Cells: A normal subject, who had never been transfused and who gave no history of hepatitis or jaundice, served as the donor of group A_1 cells. Two normal group O recipients were selected whose sera were known to be hemolytic for group A_1 red cells. Hemolytic activity was determined by titration of fresh serum in saline, without addition of complement; one volume of serum or serum dilution was incubated for one hour at 37 C. with one volume of a 1 per cent suspension of three times washed cells freshly obtained from the A_1 donor. After incubation, the contents of the tube were gently mixed, centrifuged for two minutes at 1,000 R.P.M. and then examined visually for hemolysis. For the *in vivo* studies, freshly drawn group A_1 cells were diluted in sterile pyrogen-free saline so that exactly 5 ml. of the cell suspension would contain the desired volume of red cells. The amount of hemoglobin contained in the injected red cell suspension was calculated on the basis that 1 ml. of donor cells contained 350 mg. of hemoglobin (donor's whole blood hemoglobin

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concentration = 16.8 Gm. per 100 ml., hematocrit 48 per cent). On the day of the study, the fasting recipient's *plasma volume* was measured using Evans Blue dye, and the *total blood volume* was calculated employing the venous hematocrit, suitably corrected for plasma trapping[3] and for the body:venous hematocrit ratio.[4] A blood sample was drawn for determination of the baseline plasma hemoglobin concentration and then 5 ml. of suspension of group A_1 cells was injected rapidly. A blood sample was drawn 90 seconds after the injection and subsequent samples were collected at various time intervals thereafter for determination of the recipient's plasma hemoglobin concentration.

II. Special Studies Following Transfusion of Blood Stored for 21, 32 and 42 Days: Five hundred ml. of blood was collected by gravity from each of two normal volunteers. The blood from the first donor was drawn into a conventional plastic bag** containing 75 ml. of acid citrate dextrose solution (ACD U.S.P. Sol. A) and was stored in the Barnes Hospital Blood Bank for 21 days prior to retransfusion to the original donor. The blood from the second donor was collected into 75 ml. of ACD solution contained in the primary plastic bag of a double bag unit.† After thorough mixing, half the contents were allowed to flow into the connected dry bag, and the units were separated and stored in the Blood Bank refrigerator for 32 and 42 days respectively prior to retransfusion to the original donor. On the day of the transfusion, the fasting recipient's blood volume was determined as described above. The post-transfusion destruction of nonviable stored cells was measured employing chromium[51], essentially as described by Ebaugh and coworkers.[7] Approximately 50 μc. of Cr^{51} as sodium chromate was added to the bag; the contents were mixed well and allowed to incubate at room temperature for 50 minutes. One hundred mg. of ascorbic acid was then added, and an aliquot of the well-mixed blood was removed for determination of hematocrit, whole blood and plasma radioactivity, and plasma hemoglobin concentration. The volume of the blood administered was determined from the weight of the bag before and after transfusion, suitably corrected for the specific gravity of the blood. Prior to transfusion and at intervals after completion of transfusion blood samples were collected for determination of tagged cell survival, plasma radioactivity, plasma hemoglobin concentration, and serum bilirubin concentration. Expected 100 per cent survival of the transfused cells was estimated as:

$$\text{Expected counts in red cells per ml. of recipient's blood} = \frac{\text{Total counts injected as tagged cells}}{\text{Recipient's blood volume (ml.)}}$$

III. Studies on Routine Blood Transfusion. *Stored Blood:* Five hundred ml. units of stored blood were obtained either from the general supplies in the Barnes Hospital Blood Bank or from the St. Louis Chapter of the American Red Cross. The former were collected by gravity into plastic bags containing 75 ml. of ACD solution (U.S.P. Sol. A); the latter were collected by vacuum into glass bottles containing 110 ml. of ACD solution (U.S.P. Sol. B). All blood was stored continuously in refrigerators which were equipped with constant temperature recorders and which remained at 5° ±2 C. throughout the period of storage. Immediately prior to transfusion, the donor blood was thoroughly mixed and an aliquot removed for determination of hematocrit and plasma hemoglobin concentration. The volume of blood transfused was determined by weight as described in the preceding section. *Recipients.* The recipients were in-patients

** Blood Pack (JA-2C) and
† Blood Double-Pack Unit (JD-2), Fenwal Laboratories, Somerville, New Jersey.

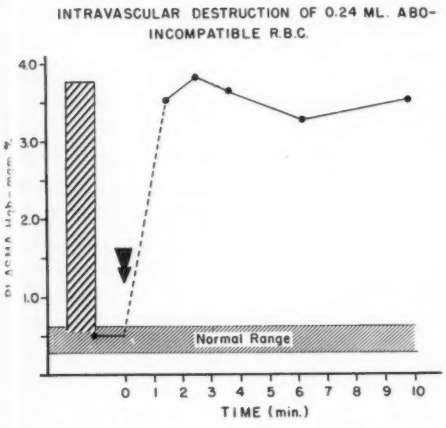

FIG. 1. Intravascular destruction of 0.24 ml. of A_1 red cells in a group O recipient. The vertical bar represents the elevation in the recipient's plasma hemoglobin concentration that would be expected if all the injected cells underwent rapid intravascular hemolysis. Note that essentially total intravascular lysis of the injected cells occurred within 1.5-3.0 minutes following injection.

at Barnes Hospital. Blood samples for hematocrit and plasma hemoglobin determination were collected from the recipients immediately prior to and immediately upon completion of each transfusion. Actual measurement of each recipient's blood volume was not attempted. Blood volume was estimated on the basis of 70 ml. per kg. of the recipient's normal body weight and plasma volume was calculated by multiplying the value for total blood volume by one hundred minus the hematocrit corrected for plasma trapping[3] and body/venous hematocrit ratio.[4]

IV. General Methods: All blood samples obtained for determination of the recipients' plasma hemoglobin concentration were drawn by a recently described method[9] designed to minimize artifactual hemolysis incident to blood collection. *Hematocrits* were determined in Wintrobe tubes spun at 2,200 R.P.M. for 55 minutes in a centrifuge of radius 25 cm. to the bottom of the centrifuge cups. *Radioactivity* of liquid samples was measured in a well-type scintillation counter. *Plasma hemoglobin* was measured by the method of Crosby and Furth[6] for concentrations greater than 4 mg. per 100 ml. and by the method of Hanks and co-workers[9] for concentrations below 4 mg. per 100 ml. *Total serum bilirubin* was measured by the method of Malloy and Evelyn.[12]

Results

***In Vivo* Destruction of ABO-Incompatible Red Cells:** Two studies were performed to illustrate the extent to which the rapid intravascular hemolysis of small volumes of donor red cells could be clearly detected by observing changes in the recipient's plasma hemoglobin level. Figure 1 illustrates what occurred when 0.24 ml. of washed A_1 cells was rapidly injected into a group 0 subject whose serum was known to contain a moderately strong anti-A hemolysin. The vertical bar indicates the rise in the recipient's plasma hemoglobin concentration that would be expected if all of the injected red cells were rapidly lysed within the circulation (based on the measured plasma volume and the known total hemoglobin content of the injected

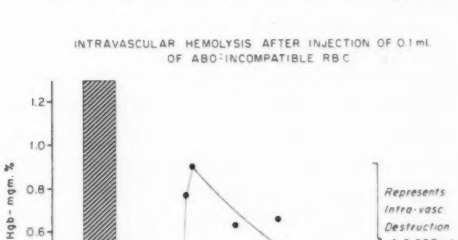

FIG. 2. Intravascular hemolysis after injection of 0.1 ml. of A_1 red cells into a group O recipient. Compare with Figure 1 but note difference in time scale. On this occasion, intravascular destruction of only 0.065 ml. of red cells evoked a four-fold rise above the recipient's baseline plasma hemoglobin level.

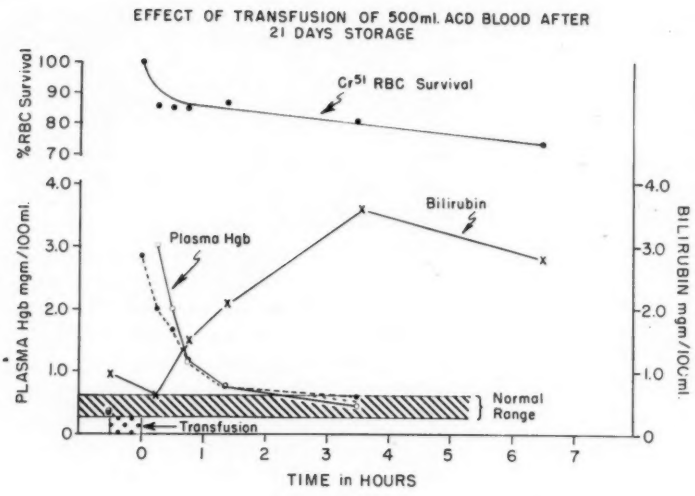

FIG. 3. Changes in the recipient's plasma hemoglobin level (o—o) after autotransfusion of 500 ml. of 21-day stored blood. The broken line (●---●) illustrates the effect of infusing the same amount of hemoglobin as was present in the *plasma* of the unit of stored blood. There is no evidence of intravascular destruction of the nonviable stored red cells which disappeared from the circulation within the first hour following transfusion.

cell suspension). It is apparent that essentially complete intravascular hemolysis of the injected cells did take place within the 90 seconds that elapsed between injection and collection of the first blood sample. Note that the lysis of only 0.24 ml. of red cells provoked a seven-fold rise above the recipient's baseline plasma hemoglobin level. A similar study carried out in a second volunteer is illustrated in Figure 2, but here the volume of injected A_1 cells was only 0.1 ml. Again there was a rapid rise above the recipient's baseline plasma hemoglobin level, the peak value being obtained at three minutes after injection. When the height of the plasma hemoglobin peak is compared with the height of the vertical bar, it is evident that on this occasion 65 per cent of the expected rise was observed, thereby indicating rapid intravascular hemolysis of only 0.065 ml. of donor red cells. Again it should be stressed that destruction of this small volume of red cells was clearly demonstrable by a four-fold increase in plasma hemoglobin concentration above the recipient's baseline level. It is not clear why the magnitude of intravascular hemolysis was less impressive in the second volunteer than in the first, a finding especially surprising in the light of their apparently comparable serum hemolysin titers (Table 1). Unfortunately, it was not possible to compare the two recipients' sera with respect to other characteristics of anti-A activity (e.g. saline, albumin and serum agglutinins, indirect antiglobulin tests). It may have been that the characteristics of the anti-A antibody in the second subject led to hepatic and splenic filtration of a proportion of the cells before intravascular hemolysis was able to take place.

Special Studies on Transfusion of Blood Stored for 21, 32 and 42 Days: As described under Methods, 500 ml. of blood was drawn from a normal volunteer, stored for 21 days under conventional blood bank

TABLE 1. *Anti-A Hemolysin Titers of Group O Recipients of 0.24 and 0.10 ml. of A_1 Red Cells*

Group O Volunteers	Hemolysis of A_1 Cells				
	Dilutions of Group O Serum				
	1/1	1/2	1/4	1/8	1/16
N. C. (Fig. 1)	C*	C	50%	tr.	neg.
T. B. (Fig. 2)	C	C	50%	tr.	neg.

*C = complete hemolysis

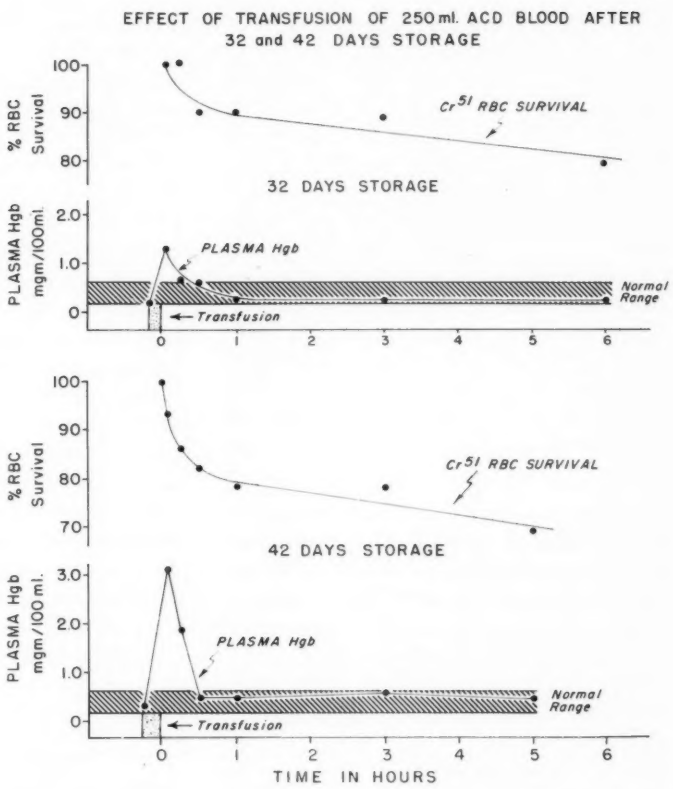

FIG. 4. Changes in the recipient's plasma hemoglobin level after autotransfusion of 250 ml. of blood stored for 32 and 42 days. As in Figure 3, the post-transfusion elevations in plasma hemoglobin concentration could be accounted for by hemoglobin present in the transfused *plasma*. There was no evidence for intravascular hemolysis of nonviable stored red cells, even in the blood stored for six weeks. The 24 hour survivals were 69 per cent for the 32-day stored blood and 45 per cent for the 42-day stored blood.

conditions, and then, after tagging with Cr^{51}, was retransfused into the original donor. The results are illustrated in Figure 3. The survival of stored cells is shown at the top of the Figure and indicates that approximately 15 per cent (i.e. 30 ml.) of the red cells were destroyed within one hour of completion of transfusion. The recipient's plasma hemoglobin concentration shown by the solid line and open circles, rose from a pre-transfusion level of 0.35 mg. per 100 ml. to 3 mg. per 100 ml. immediately following transfusion and declined to normal over the succeeding 90 minutes. Based on the recipient's measured plasma volume and the known volume and hemoglobin concentration of the transfused donor plasma, it was calculated that essentially all of the recipient's post-transfusion plasma hemoglobin elevation could be attributed to the hemoglobin present in the transfused donor plasma. To confirm this supposition, on the following day an amount of free hemoglobin identical to that present in the donor's plasma was infused over a comparable time interval; the recipient's plasma hemoglobin concentration showed an almost identical pattern, indicated by the interrupted lines and solid circles.

In order to determine whether a greater proportion of intravascular lysis of nonviable cells would occur if the period of storage were extended beyond the conventional daing period of three to four weeks, a similar study was carried out in another volunteer subject as illustrated in Figure 4. Five hundred ml. of blood was drawn

into ACD and divided into two aliquots, one of which was retransfused after 32 days of storage, the other after 42 days of storage. The results are entirely similar to the findings in Figure 3. Again the transient elevations in the recipient's post-transfusion plasma hemoglobin levels can be entirely accounted for by the hemoglobin present in the transfused donor plasma, and it is not necessary to postulate the intravascular destruction of nonviable compatible donor cells, even after six weeks of storage under conventional blood bank conditions. It is of interest that the 24 hour survival of the 32 day stored blood was 69 per cent, as compared to 45 per cent for the blood stored for 42 days.

Studies on Routine Transfusions of Banked Blood: Aliquots of donor blood were removed from the storage container immediately prior to transfusion so as to permit measurement of the amount of hemoglobin that was administered in the donor's plasma in the course of 12 transfusions given to eight hospitalized patients for the treatment of chronic anemia. In each instance an estimate was made of the recipient's plasma volume to permit a prediction of the rise in recipient's plasma hemoglobin concentration that could have been "expected" as a result of the hemoglobin present in the transfused plasma. The results are summarized in Table 2, along with the results of the studies illustrated in Figures 3 and 4. In every instance, the observed rise in the recipient's plasma hemoglobin concentration was less than "expected," with considerable variation in the discrepancies encountered among individual transfusions. When the discrepancies were analyzed with respect to the length of storage of the blood prior to transfusion (Table 3), no relationship could be demonstrated. Since it was recognized that clearance of transfused plasma hemoglobin must have been going on

TABLE 2. *Changes in the Recipient's Plasma Hemoglobin Concentration Following Transfusion of Stored Blood*

Donor blood			Recipient			
Transfusion No.	Days stored	Total Hgb. in infused plasma (mg.)	Initials	Est'd.* plasma volume (ml.)	Rise in post-transfusion plasma Hgb. conc. (mg. per 100 ml.)	
					Expected†	Observed
1	3	14	G. P.	2960	+0.47	0
2	5	70.5	T. J.	2770	+2.54	+2.00
3	5	64	L. C.	3350	+1.91	0
4	8	18	C. Z.	3900	+0.46	+0.27
5	9	30	C. Z.	3900	+0.77	+0.11
6	10	143	L. P.	2850	+5.02	+1.56
7	10	210	L. P.	2850	+7.37	+0.60
8	12	71	R. H.	3560	+1.99	+0.83
9	18	317	F. C.	3250	+9.75	+1.00
10	18	61	F. C.	3100	+1.97	+0.65
11	19	20	L. P.	2970	+0.67	+0.14
12	19	16	L. P.	3000	+0.53	+0.07
13 (Fig. 3)	21	114	G. H.	2870**	+3.97	+2.70
14 (Fig. 4)	32	37	M. A.	3120**	+1.19	+1.13
15 (Fig. 4)	42	96	M. A.	3070**	+3.12	+2.82

* Based on Total Blood Volume = 70 ml./kg. normal body weight (see Methods).

** Volumes actually measured by Evans Blue Dye Method.

† Expected from transfusion of hemoglobin present in the donor plasma.

TABLE 3. *Relation of Observed Elevation of Recipient's Post-Transfusion Plasma Hemoglobin Concentration to Age of Stored Blood*

Length of storage	Observed elevation as % of rise expected from transfused donor plasma (mean)
0 - 7 days	27
8 - 14 "	31
15 - 21 "	29

throughout the time required for administration of the blood, the discrepancies were examined with respect to the time required for administration of the transfusions (Table 4). As anticipated, an inverse correlation was clearly apparent: the less the time required for transfusion, the more nearly did the recipient's post-transfusion hemoglobin concentration approach the theoretical value "expected" on the basis of the hemoglobin present in transfused plasma.

Discussion

The mean normal value for plasma hemoglobin concentration in the human subject has recently been redefined as 0.31 mg. per 100 ml. (range 0.16 to 0.58),[9] which is approximately one-tenth the previously accepted normal value.[5] In the same study,[9] it has been shown that in the resting subject, the plasma hemoglobin level is remarkably stable, fluctuating less than ± 0.1 mg. per 100 ml. over a period of hours. Further studies[1] have determined that the plasma hemoglobin concentration is not provoked outside the normal range by sleep, ordinary daily activity, diet, vascular stasis, increased cardiac output, fever, menstruation, pregnancy, labor or parturition.

All of the above developments have opened the way to more precise study of subtle hemolytic phenomena in which relatively small changes in plasma hemoglobin concentration might be expected to occur. For example, if one considers that 1 ml. of red cells contains approximately 360 mg. of hemoglobin and if one accepts 3,000 ml. as a reasonable figure for an adult's plasma volume, it will be apparent that the prompt intravascular hemolysis of 1 ml. of red cells should raise the plasma hemoglobin concentration by $\frac{360}{30} = 12$ mg. per 100 ml., which represents an almost forty-fold rise above the mean normal value of 0.31 mg. per 100 ml. It should therefore be possible to detect accurately the destruction of volumes of red cells considerably smaller than 1 ml.; Figures 1 and 2 illustrate the extent to which this may be accomplished. Viewed against this background, Figures 3 and 4 provide useful quantitative information about what may be expected to occur to the recipient's plasma hemoglobin concentration following transfusion of stored blood. Although interpretation of the results is somewhat complicated because of the hemoglobin clearance that inevitably goes on during the time required for administration of the transfusions, it is evident from the Figures and from Table 2 that essentially all of the observed post-transfusion elevations in plasma hemoglobin concentration can be attributed to hemoglobin in the donor plasmas. In the special studies illustrated in Figures 3 and 4, the intravascular hemolysis of as little as one per cent of the transfused red cells that disappeared in the first hour should have caused further elevation of the recipients' plasma hemoglobin levels to almost twice the observed values, which

TABLE 4. *Relation of Observed Elevation of Recipient's Post-Transfusion Plasma Hemoglobin Concentration to Time Required for Transfusion*

Time required for transfusion	Observed elevation as % of rise expected from transfused donor plasma (mean)
0 - 30 minutes	57
31 - 70 "	35
71 - 95 "	20

should have been clearly detectable under the conditions of the study. Two related generalizations appear justified: (1) for practical purposes, the destruction of transfused nonviable stored red cells does not occur by intravascular hemolysis, and (2) the rise in the recipient's post-transfusion plasma hemoglobin level will depend on the amount of hemoglobin present in the donor plasma and will always be less than the maximum "expected" rise, in inverse relationship to the time required to administer the transfusion.

The rise in recipient's plasma hemoglobin that may be expected following a single transfusion of banked blood will obviously be small. Under presently employed methods of blood collection and storage the plasma hemoglobin concentration of 21 day stored blood will generally be in the range of 40-60 mg. per 100 ml.[2, 8, 15, 16, 17] (i.e. 120-180 mg. of hemoglobin in 300 ml. of transfused plasma). The results in Table 2 indicate that for blood stored up to 21 days the observed post-transfusion elevations in recipients' plasma hemoglobin concentration did not exceed 2.7 mg. per 100 ml. (in fact 10 of 13 did not exceed 1.0 mg. per 100 ml.). It is conceivable that if multiple transfusions of stored blood were administered over the course of a few hours, the body's mechanism for extravascular removal of nonviable cells might be overwhelmed and that increased intravascular destruction would then occur. Mollison[14] has apparently observed such a phenomenon in studies on rabbits. The present investigation does not provide adequate data on this point, but on three occasions (Table 2, transfusions no. 4 plus 5, 6 plus 7 and 11 plus 12) 1 liter of stored blood was transfused within three and one-half hours. The recipients' plasma hemoglobin concentrations immediately upon completion of the transfusions were 0.55, 1.33 and 0.75 mg. per 100 ml., respectively.

The information obtained in the present investigation provides a frame of reference within which to evaluate hemolytic phenomena occurring in patients who require transfusion. Patients' plasma hemoglobin levels which are elevated more than 5 mg. per 100 ml. immediately following the transfusion of one or two units of blood are almost certainly indicative of an active hemolytic process and cannot be considered a normal accompaniment of the transfusion of stored blood. Against this background, it should be possible to study in the transfused patient the possible hemolytic effects of a variety of therapeutic agents and procedures, and to make valuable comparisons between currently accepted and newly developed methods for blood preservation. Another area in which the results of the present study may prove useful is in the investigation of certain obscure transfusion reactions. It is widely accepted that if a patient has a symptomatic transfusion reaction and the plasma from a freshly drawn blood sample is not visibly pink or red, it can be safely assumed that serious intravascular hemolysis, such as is often observed with ABO-incompatibility, has not occurred. However, it has been shown[10,11] that *in vivo* incompatibility related to incomplete antibodies, such as anti-Rh, may be accompanied by a small rise in the recipient's plasma hemoglobin concentration, even though the majority of the incompatible donor cells are destroyed extravascularly. Since hemoglobin at a concentration of 25 mg. per 100 ml. of plasma is barely detectable by gross visualization, even to the experienced observer, a modest amount of immune intravascular hemolysis (significant in terms of the findings in the present study) would almost certainly be overlooked during the routine investigation of a transfusion reaction. It may be hoped that in the future actual measurement of the recipient's post-transfusion plasma hemoglobin level will prove a useful adjunct in studies of otherwise "unexplained" transfusion reactions.

Summary

1. Newly developed methods for blood sampling and hemoglobin measurement were applied to the study of changes in plasma hemoglobin concentration in the recipients of transfusions of stored blood.

2. The sensitivity of the methods for demonstrating the intravascular lysis of small volumes of red cells (0.065 and 0.24 ml.) were illustrated in group O volunteers who were given infusions of group A_1 red cells.

3. Studies following the transfusion of blood stored up to 42 days revealed that the small rises observed in the recipients' plasma hemoglobin concentrations could be accounted for by the hemoglobin present in the transfused donor plasma. It was concluded that there was essentially no intravascular lysis of the nonviable cells present in transfused stored blood.

4. The implications of these findings for the study of hemolytic processes in transfused patients are discussed.

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Change of ABO Blood Type in a Patient with Leukemia *

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THE SUBGROUPS of A are A_1, A_2 and A_3, the latter being rare. These subgroups, validated by family and population data,[4, 12] are determined by alleles of A with the following order of dominance, $A^1, > A^2, > A^3 > O$. Exceptional A types have been encountered and termed A_4, A_5, A_o, A_x, A_m and A_g.[5] Some require further study, but A_m and A_g seem to be determined by modification of the expression of normal *A* genes.

Weiner, Lewis, Moores, Sanger and Race[11] showed that A on the red cells could be suppressed by a recessive allele, *y*, at a locus independent of the ABO genes, but A in the saliva was affected very little. An interaction effect between A alleles and the *yy* genotype results in suppression of A on the red cells, and an unusual ABO type, A_m. Present data do not permit an opinion concerning the role of *Y*, the allele of *y*.

Another exceptional type is A_g. This was first described by van Loghem, Dorfmeier and van der Hart[10] in a patient with acute leukemia. Subsequently Stratton, Renton and Hancock[8] reported a similar patient, who had "hypoplastic anemia" and shortly afterwards presented with acute leukemia. The red cells of both these patients, although typed previously without difficulty as type A, failed to agglutinate or agglutinated only weakly with anti-A, although anti-A could be absorbed by these cells and then recovered in heat eluates. Both cases were non-secretors of ABO substances.

We have recently encountered a similar instance of a patient with acute myeloblastic leukemia, where blood type A changed to type A_g. This patient was a secretor of A and H substances, and in the terminal phase of his leukemia reverted to subtype A_1.

Case Report

Mr. N.V., a 45-year-old white male was first seen in The Mount Sinai Hospital in July 1958 with acute myeloblastic leukemia. The patient was treated with amethopterin followed by 6-mercaptopurine; a good remission lasting for six months was obtained. Final relapse and death occurred in August 1959. The clinical details are shown in Figure 1.

From military service records it is known that the patient had been blood typed in 1944 as type A. In October 1958 the patient's red blood cells were observed to be type O, but no anti-A could be demonstrated in his serum. He was uneventfully transfused with five units of type O blood. The patient was a secretor of A and H substances. His wife, two children and a brother all proved to be type A_1 and all except the brother were secretors of A and H substances (Table 1).

Absorption and elution tests with high titer type O serum yielded anti-A from the patient's red cells, but similar tests with high titer type B serum were negative. Agglutination of the patient's red cells by anti-A sera of type O, type B, or other source, was never observed during his clinical remission, and the red cells of the patient were agglutinated by anti-H reagents (human as well as extract Ulex europeus) to the same degree as

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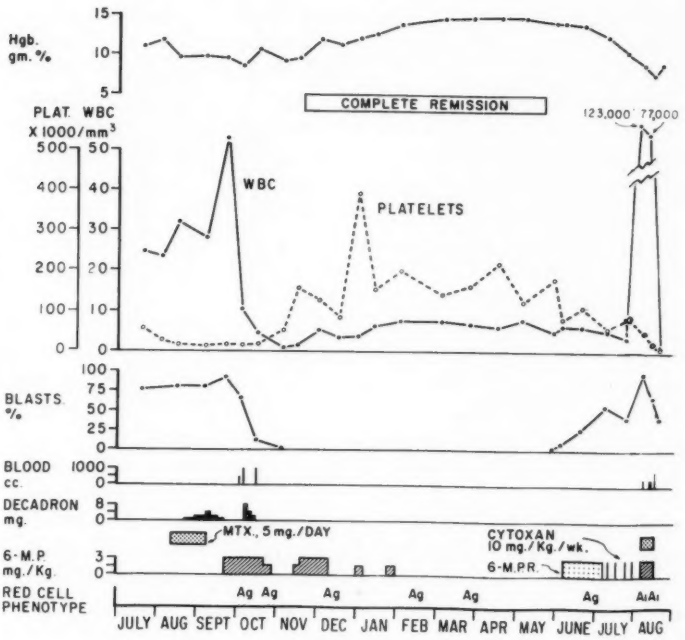

FIG. 1. Clinical Course of Mr. N.V. From October 1958 until June 1959, the red cells lacked A antigen and the phenotype was designated six times as A_g. Suddenly in August 1959, the patient's red cells were observed twice to behave as subtype A_1.

control type O cells. During the terminal stage of the disease the ABO blood type reverted to A_1, and the patient's red cells could no longer be agglutinated by anti-H reagents. The A_1 subtype status was clearly shown with saline extracts of the seeds of Dolichos biflorus, and the patient was now uneventfully transfused with five units of type A donor blood. Atypical isoagglutinins were not observed, although saline, ficin and antiglobulin tests were performed at frequent intervals using O and A_1 test cells. Repeated direct antiglobulin tests were also negative.

In the final period of the patient's illness, a recurrence of leukemic cells in the peripheral blood was followed shortly by a fall in hemoglobin. In six weeks, the blood type changed from A_g to A_1, while the hemoglobin fell from 12 to 6 Gm. In the same period reticulocytes averaged < 1 per cent indicating some impairment of erythropoesis. Two alternatives exist, neither of which can be substantiated: (1) in the 6-week interim between A_g and A_1 blood typing observations, all A_g cells disappeared from the circulation and were replaced by type A_1 cells; (2) the change from A_g to A_1 was relatively sudden and affected mature peripheral erythrocytes in addition to erythroid precursors. Since all cells were type A_g at the end of June 1959, and all cells were type A_1 (certainly > 98 per cent) in early August, the latter possibility appears to be the more likely. A mixed population of cells was never observed.

Discussion

The loss of red cell A reactivity (type A_g) was first observed during the early

TABLE 1. *Comparative Saline Agglutination Inhibition Values of the Saliva of Mr. N.V. and of his Relatives*

	A anti-A	H anti-H	Le^a anti-Le^a
N. V., patient	1:320	1:40	1:80
M.V., wife	1:320	1:40	1:80
B.V., son	1:160	1:40	1:40
F.V., daughter	1:320	1:40	1:40
A.V., brother	0	0	1:320

stages of acute myeloblastic leukemia and the altered blood type persisted throughout a lengthy clinical and hematological remission. The blood type change was restricted to the red cells however, because the saliva contained normal amounts of A as well as H substances; earlier case reports dealt only with non-secretors of ABH.[8, 10] During the short terminal stage of his illness the patient's red cells reverted to their normal status, because they then agglutinated readily with both anti-A and anti-A_1 reagents, but no longer agglutinated with anti-H. This, too, differs from earlier reports.

A second case in the report of van Loghem, et al,[10] and a case described by Dodd and Gilbey,[1] might have been other examples of the *yy* modification of blood type A (A_m). On the other hand, a patient with chronic myelocytic leukemia, described as A_m by Wiener and Gordon,[13] might have been another example of acquired loss of red cell A reactivity.

Complete loss of the normal A character of red cells is not the only abnormal ABO serologic reaction that has been observed in patients with leukemia. Salmon, Borin and Andre[6] reported a patient with a double population of red cells, 75 per cent subtype A_1 and 25 per cent type O. Salmon, Andre and Dreyfus[7] later reported another case of type A having 20 per cent of red cells which they described as "weak A."

A case of acute monoblastic leukemia in which only 2 per cent of the red cells could be agglutinated with potent anti-A sera, was described by Gold, Tovey, Benny and Lewis;[3] the remainder of the red cells of this patient were agglutinated very strongly by anti-H but poorly by anti-A. Later during a period of clinical improvement, 35 per cent of the red cells could then be agglutinated with anti-A sera. There was no agglutination at any time with Dolichos extract anti-A_1.

Disappearance of A antigen from the cells as a result of somatic mutation during the course of leukemia,[7] does not easily explain the course of events in the case now reported. Conditions early in the leukemia, and continuing throughout a lengthy remission, would have to provide marked selective advantages for the mutant erythroid precursors to account for an absence of all type A cells. Our case, in addition, would demand a complete reversal of such conditions along with restoration of somatic A competence, all in a period of less than six weeks.

Prior to leukemia, this patient's erythrocytes presumably possessed A_1 but not H antigenic structural groupings. During leukemia and leukemic remission, the patient's red cells lacked A_1 but possessed H. And finally, in the terminal phase of leukemia, the peripheral red cell population of this patient lost their acquired H character while regaining A_1. The H and A_1 antigenic determinants of erythrocytes thus, even in this patient, consistently bear an alternative relationship to each other. When either one is fully expressed, the other is absent. This is not true however, of secreted mucopolysaccharides which, in ABH secretors, including the case described, consistently bear H even if they bear A_1.

The serologic findings in the present case report, except for reversibility of the ABO erythrocytic antigens, are very similar to those reported for A_m.[11] The genotype *AB yy*[11] has been observed to lack A but not B on the erythrocytes, whereas A, B and H were encountered in secreted mucopolysaccharides. Dunsford[2] has observed loss of erythrocytic A antigen in a type AB patient with leukemia. Thus the leukemic effect on the erythrocytic A antigen further resembles the genetic interaction effect of *yy*.

Stratton and Renton[9] observed erythroid elements of the bone marrow in type A_2 to lack A antigen just as the peripheral mature red cells. Therefore A is not lost

or covered in consequence to erythrocytic aging.

It is not possible to state at this time whether some leukemia (or leukemia therapy) and the *yy* state interrupt the development of erythrocytic A antigen, or accentuate H at the expense of erythrocytic A at the red cell surface. The effect is an interesting one however, and points to the complex physiologic pathway lying between the *A* gene and its ultimate expression on the red cell.

It would be of considerable importance to observe the blood typing characteristics of type A_1 donor cells in a type A_g recipient, to determine the integrity of the A_1 antigenic character of the donor cells.

Summary

Another instance of a change in ABO blood type has been described in a patient with acute myeloblastic leukemia. Blood type A disappeared and the patient assumed a blood type which lacked A on the red cells (A_g), with serologic properties similar to those described for a genetically determined lack of erythrocytic A expression (A_m). This patient suddenly reverted to A_1, losing erythrocytic H in the process, but only during the final relapse of the leukemia.

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A-B-O and **hr′** Hemolytic Disease in the Same Family *

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WELL documented cases of more than one form of hemolytic disease in the same family are uncommon. Therefore, it seemed of interest to present a family in which both **hr′** hemolytic disease and A-B-O hemolytic diseases were encountered.[1, 2]

Case Report

Our first contact with this family occurred in 1956 with the birth of the third child (cf Table 4). Labor and delivery had been at term and uneventful. However, early onset of jaundice and anemia and a positive direct antiglobulin test led to a diagnosis of hemolytic disease, and one of us (A.S.W.) was called into consultation.

The mother had been transfused twice in 1942, and again once in 1954. The first pregnancy (1950) had resulted in a miscarriage at three months. The second pregnancy (1951) had terminated as a normal full term delivery—this child had no neonatal jaundice and was living and well. Labor and delivery for the third pregnancy (1952) had been at term and uneventful; however, this child had mild neonatal jaundice (by history) and now had cerebral palsy. The fourth pregnancy (1956) produced the patient in question.

The clinical summary strongly suggested the diagnosis of hemolytic disease. However, the mother and all other members of the family were Rh positive. The newborn infant was group A, Rh positive, and positive for the **hr′** factor while the mother was O, Rh positive, negative for the **hr′** factor and possessed antibodies for this factor in her blood serum. Therefore a diagnosis of **hr′** hemolytic disease was made. Moreover, since the baby was group A and the mother O, the possibility of A-B-O hemolytic disease was also considered, but the subgroup of the child later proved to be A_2, and subgroup A_2 babies are virtually exempt from A-B-O hemolytic disease.[2, 6] However, the possibility cannot be entirely excluded that the baby had both **hr′** and A-B-O hemolytic disease.

The mother had probably first become sensitized to the **hr′** factor at the time of her transfusions in 1942, but it is possible that the miscarriage also played a role. Since 80 per cent of the population has the **hr′** factor, the likelihood was high that at least one of the two donors was **hr′** positive. The first child was subgroup A_2, Rh positive and negative for the **hr′** factor. This child had no neonatal jaundice and is now living and well. The second child was not as fortunate; he was subgroup A_1 and positive for the **hr′** factor. This child did have neonatal jaundice and now has cerebral palsy. There is no way of determining whether or not this child had hr′ and/or A-B-O hemolytic disease. The simultaneous presence of A-B-O incompatibility has been shown to interfere with the evolution of $\mathbf{Rh_o}$ or **hr′** sensitization by preg-

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In this paper, in conformity with the recommendation of the Committee on Medicolegal Problems of the American Medical Association, symbols for agglutinogens and phenotypes are printed in regular type, symbols for blood factors and their corresponding antibodies in **bold face** type, while symbols for genes and genotypes are printed in *italics*.

TABLE 1. *Results of hr′ Antibody Tests When Third Child Was Born*

Serum of	Titer by method of			
	Agglutination	Albumin-plasma conglutination	Anti-globulin	Ficinated cells
Mother	8	44	300	400
Baby	0	3	12	112

ancy, but in this particular case it did not, because the original source of the hr′ sensitization was probably the blood transfusions. In fact it would be possible for the damaging effect of sensitizations within the two systems to be superimposed.

The patient was subgroup A_2, hr′ positive and evidently had hr′ hemolytic disease. Table 1 compares the titers of hr′ antibody in the serum of the mother and of the child. The saline agglutination method detects the "bivalent" form of antibody which has greater molecular size and does not pass the placental barrier, which explains its absence from the cord serum.[3, 4] However, the other form (or forms) of antibody detected by the other three methods listed in the Table traverse the placenta readily when the baby's cells are compatible, and the titers in both mother and baby are generally equal. In this case, however, the titers in the baby were considerably lower because most of the antibody had been absorbed by the fetal hr′ positive red cells. The baby was treated by exchange transfusion, with hr′ negative blood, and is now alive and well.

The mother then became pregnant for the fifth time. In Table 2 are given the results of the hr′ antibody titrations chronologically arranged from the fourth pregnancy at term until the postpartum period of the fifth pregnancy. This Table spans a period of two years. The maternal antibodies were quite high when the child with hr′ hemolytic disease was born (4th pregnancy). Between the 4th and 5th pregnancy there was an interval of one year during which time the titers declined, so that in the second month of the 5th pregnancy the titers were considerably lower. Throughout the 5th pregnancy the titers remained relatively low.

The failure of the titer to rise during the

TABLE 2. *Summary of All hr′ Antibody Titrations on Mother's Serum*

Time of test		Titers by Method of			
		Agglutination	Albumin-plasma conglutination	Anti-globulin	Ficinated cells
4th preg.—At term		8	44	300	400
	Postpartum	3	90	—	960
5th preg.	2nd mo.	0	—	41	92
	6th mo.	0	—	22	56
	7th mo.	0	5	64	88
	8th mo.	0	20	28	96
	9th mo.	0	8	12	52
	9th mo.	0	4	14	36
	9th mo.	0	8	14	48
	Postpartum	0	3	14	44
5th preg.	Mother's Avg. titers	0	9	28	56
	Baby's titers *	0	8	16	32

* The baby's serum is included for comparison with the maternal serum.

TABLE 3. *Results of Titrations of A-B-O Antibodies of Mother's Serum*

Time of test	Titer in saline for			Titer in acacia for		
	A_1	A_2	B	A_1	A_2	B
4th preg.—At term	60	15	10	1750	950	160
5th preg. 6th mo.	120	60	15	480	160	30
7th mo.	35	40	20	640	400	120
9th mo.	80	40	10	950	400	50
At term	60	20	20	320	240	70
Avg. titer	74	40	16	600	300	68
Postpartum	240	—	20	2500	—	50

pregnancy indicated two possibilities: (1) either the fetus was **hr′** negative, or (2) the fetus was **hr′** positive, but virtually none of the fetal red cells had entered the maternal circulation and further sensitized her. As it turned out, the child was **hr′** negative, and, as shown in Table 2 as expected, the titers of **hr′** antibody in the baby and in the mother at term were approximately equal. Moreover, as expected because the child was **hr′** negative, there was no postpartum rise of the maternal titer. Despite this the cord icterus index proved to be 24 units and the baby developed jaundice soon after birth. By the following morning the jaundice was deeper—the icterus index was 75 units, the hemoglobin concentration was 15.3 grams, the red cell count was 5.3 million, and there were frequent spherocytes noted in the smear. The fact that the direct antiglobulin test was negative and that the baby was **hr′** negative ruled out **hr′** hemolytic disease, and since the baby was group A a diagnosis of A-B-O hemolytic disease was made. As expected, subsequent tests showed that the baby was subgroup A_1.

The A-B-O antibody titrations carried out on the mother are listed in Table 3. At the time when the child with **hr′** hemolytic disease was born, the maternal anti-**A** titers were quite high (4th pregnancy at term, Table 3), so that it was suspected that the child might also have A-B-O hemolytic disease. However, as already pointed out the infant proved to be subgroup A_2. During the 5th pregnancy the anti-**A** titers were somewhat lower, and remained essentially unchanged throughout the pregnancy. Despite the failure of the titers to rise, this child did suffer from A-B-O hemolytic disease. There was a specific anti-**A** titer rise in the postpartum period.

In Table 4 the blood groups and secretor status of the entire family are summarized. Everyone in the family is Rh positive and an ABH secretor. The secretor status has no apparent relationship to Rh-Hr hemolytic disease, but based on evidence pre-

TABLE 4. *Blood Groups and Secretor Types of Entire Family*

Blood of	A-B-O groups	Saliva tests	Rh-Hr types	Factor **hr′**
Father	A_1	Secretor	Rh_1rh	Pos.
Mother	O	Secretor	Rh_1Rh_1	Neg.
1st child (normal)	A_2	Secretor	Rh_1Rh_1	Neg.
2nd child (cerebral palsy)	A_1	Secretor	Rh_1rh	Pos.
3rd child (E. F., rec.)*	A_2	Secretor	Rh_1rh	Pos.
4th child (E. F., rec.)	A_1	Secretor	Rh_1Rh_1	Neg.

* E. F., rec. = erythroblastosis fetalis, recovered.

sented elsewhere,[2] the authors believe that the secretor status does play a role in A-B-O hemolytic disease—although this view is not shared by other workers. There appears to be two forms of A-B-O hemolytic disease.[2] In one, typically the milder form, the "natural" or heterogenic isoantibodies of the mother are involved and the first born incompatible baby is often affected. In the second, typically the more severe form, the first incompatible baby may escape, but is a secretor, and his secretions sensitize the mother so that subsequently born incompatible babies are affected by the mother's iso-immune antibodies. However, type 2 or the more severe form of A-B-O hemolytic disease can also affect the first born.

Since half the children are subgroup A_1 and half are subgroup A_2 the father's genotype must be A_1A_2. Therefore, any future baby in this family would have an equal chance of being subgroup A_1 or subgroup A_2.[5] Because the father is type Rh_1rh and because half of the children are **hr′** negative, it follows that he must be heterozygous for the **hr′** factor. Therefore, future children of this family would have an equal chance of being **hr′** positive or **hr′** negative. Thus, in any future pregancies in this family, only one out of four children would be expected to escape hemolytic disease, that is, a child, such as the first one, who is both subgroup A_2 and **hr′** negative.

Summary

An immunogenetic study of a family in which both A-B-O and **hr′** hemolytic disease occurred has been presented.

The father was group A, type Rh_1rh (**hr′** positive) and the mother group O, type Rh_1Rh_1 (**hr′** negative). All the children were group A, two subgroup A_1 and two subgroup A_2. Two children were type Rh_1Rh_1 and two type Rh_1rh. All members of the family were ABH secretors. The mother had been sensitized to the **hr′** factor by blood transfusion received prior to her first pregnancy, and was also strongly sensitized to the blood factor **A**. Three out of the four children of this family had hemolytic disease—two were treated by exchange transfusion and are now living and well, while the untreated child has cerebral palsy.

On the average only one fourth of future children of this family would be expected to be free of hemolytic disease, namely, those who are both subgroup A_2 and type Rh_1Rh_1.

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Survival of Chromium^{51} Labelled Red Cells in the Presence of Anti-H and Anti-A_1 *

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Introduction

IN THE decades since von Dungern and Hirszfeld[10] described the subgroups of human group A red blood cells, a number of authors have observed examples of anti-A_1 and anti-A_2 antibodies (α_1 and α_2) in the serum of individuals of group A_2, A_2B, A_1 and A_1B. Landsteiner and Levine[5] pointed out that anti-A_2 (α_2) often has anti-O activity while Watkins and Morgan[11] further divided the anti-O antibodies according to their ability to react with the "H" substance of saliva. The term anti-H has since been applied to anti-A_2 (α_2) antibodies which react with "H" substance.

Even the oldest descriptions imply that α_1 and α_2 are not exactly analogous to one another but little information is available concerning the reasons for the differences between them. In addition, very little information exists concerning the importance of these antibodies in altering survival characteristics of transfused red cells.

We recently had an opportunity to study examples of the α antibodies which developed in two patients who were subject to transfusion therapy. The correlation of the serological findings with the survival of transfused incompatible cells forms the substance of this report.

Case Reports

Case 1: H.T.T., a 58-year-old white male, was admitted to the hospital with a diagnosis of epidermoid carcinoma of the anus. He had not been previously transfused to his knowledge. A radical excision of the tumor was performed but persistent bleeding and extensive infection necessitated twenty-two 500 ml. whole blood transfusions in a period of 32 days. The patient was originally found to be A_1 Rh_o (D) positive. In addition he was M, N, S, U, P, rh′ (C), hr′ (c), hr″ (e) positive and hr″ (E), K, Fy^a and rh^w (C^w) negative. The direct Coombs test was negative and there was no autoagglutination. The transfusions given were of both A_1 and A_2 blood.

No irregular antibodies were demonstrable before surgery or on the seventh, 14th, 28th and 33rd postoperative days, but on the 38th day an antibody was found. It proved to be a saline agglutinin which was active at 37 C. (+ agglutination after 30 minutes incubation) against group O cells. The antibody agglutinated all group O cells in our red cell antigen panel. These included cells known to be negative for M, N, S, P, rh′ (C), rh^w (C^w), hr′ (c), Rh_o (D), rh″ (E), hr″ (e), Lu^a, K, Le^a, Le^b, Fy^a and Jk^a. At 22 C. the antibody gave a +++ agglutination** with group O cells and had a titer of 1:4. Forty-seven of 62 A_2 red cell samples randomly chosen were also agglutinated. At 15 C. and below, the α_2 antibody had a titer of 1:8 and weakly agglutinated A_1 cells as well as the cells circulating in the patient. After storage at −20 C. for two weeks, the antibody became weaker and lost most of its activity above 15 C. The antibody could be inhibited by group O secretor saliva† (and indeed it could be inhibited by the patient's own saliva) at all thermal ranges indicating that it had "anti-H" specificity.

* Received July 5, 1960; accepted September 20, 1960.

** All agglutination graded as by Race and Sanger.8

† Method for determining secretor status and inhibition test as described by Dunsford and Bowley.3

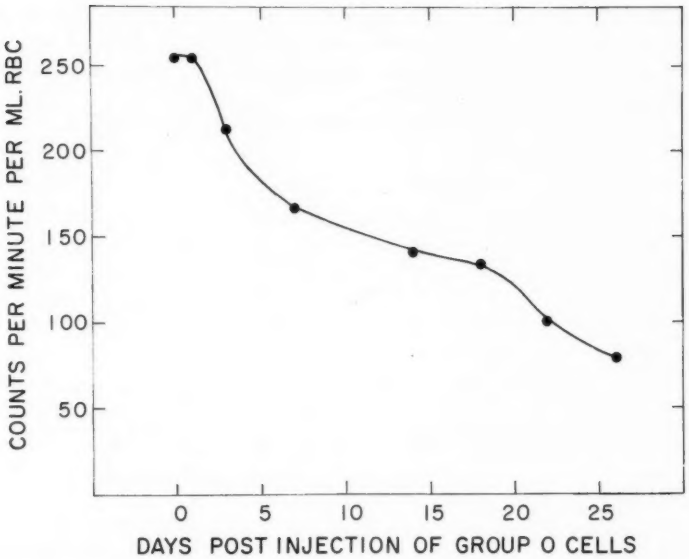

FIG. 1. Chromium[51] survival time of group O red cells transfused to a recipient with anti-H.

Since the possibility of group O emergency transfusions had been raised, a chromium[51] survival time of *in vitro* incompatible group O cells was undertaken. This *in vivo* test of compatibility was carried out during a period when the patient's hematocrit was stable and when no other transfusions had to be given. Three ml. of group O rr (cde/cde) red cells from a normal blood donor were labelled with sodium radiochromate (chromium[51]) of high specific activity according to the method of Mollison[7] and given to the patient intravenously. Red cells labelled in the same manner and given to a normal recipient without antibody being present usually have given a 27 to 32 day initial apparent half time in our hands.

Despite the fact that the patient's serum agglutinated the donor group O cells strongly *in vitro*, red cell survival during the first 24 hours was normal. Subsequent determinations revealed an overall 18 day initial apparent half-time of the chromium[51] label (Fig. 1). The titer of antibody in the patient did not fluctuate during the chromium[51] survival period.

Case 2: Patient J. W. entered the hospital for restudy of hyperparathyroidism. Two years previously a cervical and substernal exploration for parathyroid adenomas had been performed at another hospital with negative results. The patient had been grouped as AB, Rh_o (D) positive and had been given one unit of AB blood at surgery without untoward effect. No antibody was noted in his serum at that time.

On admission the patient's red cells were grouped as A_2B and serum grouping disclosed anti-A_1 (α_1). His red cells were also positive for M, N, S, P, rh′ (C) hr′ (c) Rh_o (D) hr″ (e), k and Jk^a and negative for rh″ (E), K, Fy^a and rh^w (C^w). The direct Coombs test was negative and there was no evidence of any hematological abnormality. The antibody (like the anti-H in case #1) gave a + reaction when incubated for 30 minutes at 37 C. with a 2 per cent saline suspension of A_1 red cells. The titer was 1:2 at 22 C.

The antibody did not agglutinate any group O red cells known to be positive for M, N, S, s, P, rh′ (C), hr′ (c), rh^w (C^w), Rh_o (D), rh″ (E), hr″ (e) Lu^a, K, k, Le^a, Le^b, Fy^a, Fy^b, Jk^a and Jk^b. Of 31 randomly chosen A_2 red cell samples, none were agglutinated. All of 24 randomly chosen A_1 red cell samples were agglutinated.

Since the clinical history indicated that this anti-A_1 antibody might be of "immune" origin, it was decided to do a chromium[51] survival time to learn whether A_1B cells could be used safely. The patient was given 1.5 cc. of A_1B cells from a normal blood donor labelled with 25 μc of radiochromium according to the method of Mollison.[7] There was an immediate loss of 20 per cent of the chromium[51] activity within the first ten minutes indicated by estimated blood volume values and not shown on Fig. 2. The second component of Cr^{51} disappearance then had an initial apparent half-survival time of seven and one half days (Fig. 2). The antibody titer against the infused cells was 1:2 at 22 C. prior to infusion and 1:4 15 days later but no hemolysin appeared.

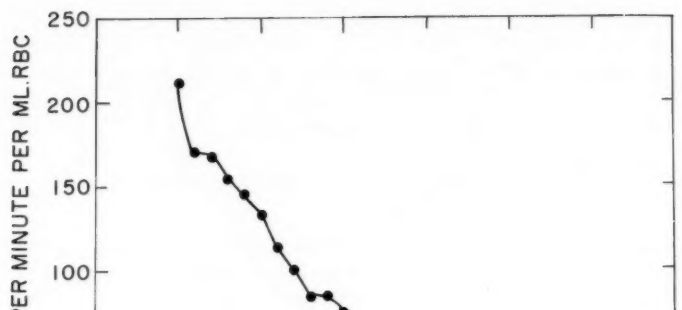

FIG. 2. Chromium[51] survival time of group A_1B red cells transfused to a recipient with anti-A_1.

Discussion

The first case is an illustration of an α_2 antibody with both anti-O and anti-A_2 activity. By Watkins and Morgan's[11] nomenclature, this antibody should be called an anti-H. To our knowledge only two transfusion complications due to α_2 antibodies have been reported and both of these were due to anti-O, not anti-H. Horvath[4] described in detail an anti-O agglutinin as the cause of a hemolytic transfusion reaction. The patient was a young mother of group A_1B who received 300 ml. of group O blood. The titer was slightly higher than in our case (1:2 at 37 C. and 1:16 at 22 C. against O cells). The author, noting that the titer fluctuated during pregnancy, concluded that the antibody was a natural anti-O which had undergone conversion to an immune form.

Recently Mollison[6] described the survival characteristics of group O erythrocytes in the presence of an α_2 (anti-O) antibody, which had similar serological characteristics to the anti-H antibody described in this report except that, like Horvath's case, it was not neutralized by secretor saliva. Again, unlike the α_2 antibody we describe here, Mollison found his α_2 to be quite destructive of group O red cells. Infused group O cells had an initial half-time of three and one-half minutes.

Perhaps the striking dissimilarities in red cell viabilities between these cases of anti-O and the one we studied can be correlated exactly with the anti-H specificity of the antibody. More data would be needed before this correlation could be drawn conclusively.

In our case 1 we do not know for certain what stimulated the activity of α_2. The stimulus might have been the sepsis at the patient's operative site or the transfusion of A_2 red cells. Although the antibody agglutinated group O red cells at body temperature it did not disastrously affect their viability and was not restimulated by *in vivo* exposure to test cells.

Chromium[51] survival times of 27-32 days have been the rule for normal individuals, but some patients seriously ill with sepsis, as this patient was, have shown shorter red cell survival times in the absence of irregular antibodies. Thus, we cannot be certain in the first case that the shortening of the survival time was due to anti-H, since

the patient's own cells might not have shown an apparent half-time much longer than 18 days. The conclusion drawn is that the survival time of the group O cells was not dangerously affected and that red cells containing the "O antigen" could be given to the patient in an emergency.

In contrast is the nature of α_1 as exemplified by our second case, where the pattern of red cell elimination was indicative of immunologic incompatibility. According to Taylor,[9] 26 per cent of A_2B people possess anti-A_1, but data are lacking concerning the mode of origin of this antibody as a natural isoagglutinin. In our case we have an example of an antibody probably stimulated by a previous transfusion and taking a form which greatly accelerated the destruction of red cells. This sort of patient should certainly not be given blood containing the A_1 antigen.

A few examples of the transfusion of the A_1 agglutinogen to patients with α_1 have been published. For example, Boorman, *et al.* pointed out that the α_1 agglutinin can be stimulated to an immune destructive form by transfusion, with good evidence for rapid elimination of transfused A_1 cells. Our case 2 illustrated again that α_1 can arise in association with transfusion, and that even when the antibody is only weakly active at 37 C., impaired red cell survival can result.

Summary

Red cell survival studies with two examples of "warm" antibodies within the ABO system are presented. One antibody was an α_2 antibody (with anti-H specificity) in a patient of group A_1. The other was an α_1 antibody (anti-A_1) in the serum of a patient of group A_2B. Although these antibodies gave very similar *in vitro* reactions, the α_2 antibody may not have affected the viability of injected group O cells, while the α_1 antibody seriously affected the survival of transfused A_1B cells.

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Special Reports

Thirteenth Annual Meeting, American Association of Blood Banks

August 21-26, 1960, San Francisco, California

THE PROGRAM was divided into administrative, technical and scientific sections. The general theme of the scientific program was set by Dr. P. Levine, who reviewed Landsteiner's contributions to human blood groups. This was an inspiring, informative and amusing introduction to the scientific program. The papers that afternoon dealt with blood groups. Sanger reviewed the Rh system and emphasized that new developments were beginning to resolve some of the inconsistencies in the DCE notation and the system it represented. The concept of the complex antigen, *ce*, and its identity as *f* were discussed. Greenwalt presented statistical evidence for linkage between secretion and the Lutheran blood groups. Shapiro discussed hr^s, a new blood factor discovered in the serum of a Bantu woman (Mrs. Shabalala). His conclusions explained some previously reported anomalous findings which had been attributed to illegitimacy. Tate, *et al.*, reported an Rh gene complex, *Dc-*, and Levine, *et al.*, presented a family with seven Vel negative (*VeVe*) members.

The next morning, Mollison reviewed some of the relationships between complement and the antiglobulin test. The nature of blood group antibodies which did and did not require C′ for detection was reviewed. The concept of a complement-like substance "complementoid" was discussed and the anti-complementary activity of stored sera was thought to be related to alterations in globulins. Methods for detecting C′ antibodies were reviewed. Immunoconglutinin (I-K), an "antibody" against complement combined in the antigen-antibody reaction was also discussed. Hackel and Boyd presented new data in studies of the biochemical structure of Rh antigens.

In the afternoon Reed, Bishop and Shafer and Bartlett discussed metabolism of erythrocytes. Shafer pointed out that late addition of inosine produces more regeneration of ATP than addition at the time of storage. The discussion which followed these papers was particularly exciting, both in the glimpse of practical application to longer storage and the increasing knowledge of the intimate habits of the erythrocyte.

Tullis, Pyle, *et al.*, Haynes and Sproul and Strumia, *et al.*, provided observations on the storage of red cells at low temperature. The experiences reported by Haynes of the successful routine operation of a hospital blood bank with blood stored for long periods at low temperature were of great interest.

The next morning opened with Dr. Mourant's discussion of the relationship of anthropology and blood groups. Hereditary blood factors in Indians in Central America and North America were described in two other papers. Fudenberg and Kunkel reported on the interesting observations made using the sera of some patients with rheumatoid arthritis defining inherited antigen sites in the red cells in man. Haberman and Krafft and Cohen, *et al.*, reported on ABO hemolytic disease of the newborn.

The next morning was devoted to a group of miscellaneous papers. Observations on the inheritance of white blood cell groups were presented by Payne and Hackel. Cassel and Chaplin described an elegant technic for the detection of minute quantities of free hemoglobin in the plasma. Swisher, *et al.*, laid to rest the erroneous idea that administration of corticosteroids could prevent reactions due to red cell incompatibility. Giblett reviewed the theoretical hazard of inter-racial blood transfusion and proved that there was no statistical validity to favor intra-racial over inter-racial transfusion.

On the whole, the meetings were excellent. The papers were interesting and a reasonable amount of critical discussion followed many of the presentations. I have not listed all the papers read. A complete list and abstracts are available in the program of the meeting.—*Julius R. Krevans, M.D.*

A Review of Landsteiner's Contributions to Human Blood Groups*

PHILIP LEVINE, M.D.**
Raritan, New Jersey

WHEN requested by your Program Committee to open these sessions with an address on the occasion of the sixtieth anniversary of Landsteiner's discovery of the blood groups, I considered this invitation as a signal honor. It is now almost a full generation since Landsteiner died in 1943. To the younger workers in blood banks and transfusion services his name is a legend and the man a mystery. The junior set and some of their older colleagues could very well ask: "What manner of man was Landsteiner, whose memory we are honoring today?" I should like to satisfy your curiosity and help you to develop a feeling about him as a personality before embarking upon the main theme—his accomplishments—at least that segment of his numerous discoveries which bind us together as members of the A.A.B.B., a description of our joint work from 1925-1932, and the impact of his scientific contributions and their bearing on the current and future status of blood groups.

Dr. Mohn as Chairman of the Program Committee, requested me to tell you the story of my work shortly after leaving Landsteiner. Further implications of Landsteiner's influence will be given this afternoon by the "better half" of the Race-Sanger* duet with little or no apology to Race. Directly or indirectly, the papers of Mollison* on Tuesday and Mourant on Wednesday are further outgrowths of Landsteiner's pioneer contributions.

After working in Landsteiner's laboratory from 1925-1932 and spending many evenings at his home preparing papers, as well as periodic visits and consultations from 1932 until his death in 1943, I can honestly state that I knew him well and apparently the Program Committee concurs.

Landsteiner was born in Vienna on June 14, 1868 of Jewish parents. His father, Leopold, was a journalist who died when Karl was six years of age. The son was utterly devoted to his mother, Fanny Hess Landsteiner, and to this day I still recall the reverence and warmth of feeling, when at the age of sixty, he spoke to me of his mother. The announcement of his marriage to Helen Wlasto was delayed until the death of his beloved parent.

In 1891, at the age of 23, Landsteiner received his medical degree in Vienna and for the next five years studied chemistry, the better to equip himself for the years ahead. He served for one year as assistant to Prof. Von Gruber and in 1899 was certified as a pathologist. Later he became assistant to Weichselbaum, discoverer of the meningococcus and to Fraenkel, co-discoverer of the pneumococcus. During this time a series of important observations were made both in pathologic anatomy and in immunity I recall Landsteiner telling me that his chief, Weichselbaum, although pleased by the younger man's contributions, was not quite enthused.

So that more time could be devoted to experiments dealing with bacterial agglutination and lysis, he quickly disposed of his routine obligations and stated duties as pathologist and teacher of morbid anatomy. It was during this period that he recorded but quickly dismissed his observations on blood groups. His mind was already oriented toward basic mechanisms of immunologic specificity but little could be accomplished without resorting to the symbolic thinking of Ehrlich.

Notable contributions which flowed freely from his active and fertile mind were interrupted by World War I. There followed a series of frustrations, loss of facilities in the laboratory, personal deprivation and virtual starvation. In 1919, with the aid of friends, he was given space in a corner of a laboratory in a small Roman Catholic Hospital in The

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* Keynote address at the 13th Annual Meeting of the American Association of Blood Banks, San Francisco, August 22, 1960.

** Director, Division of Immunohematology, Ortho Research Foundation.

* Ed. Note: Drs. Race and Sanger received the Landsteiner Award in 1957. Dr. Mollison received the Landsteiner Award on Wednesday, August 24, 1960.

Hague. Several years ago I visited this hospital and saw a small plaque in the corner of a laboratory indicating that here Landsteiner worked from 1919-1921. He earned his keep during this time by doing routine laboratory tests, performing autopsies and carrying out fundamental studies on haptens, anaphylaxis, heterogenetic antigens and allergy. For extra income he made old tuberculin for Blumberg, a pharmacist whose shop I saw in 1950. Rous in his excellent appreciation of Landsteiner published in Obituary Notices of the Fellows of the Royal Society, March 5, 1947, tells how a friend in Holland sent word to the United States of Landsteiner's plight. An offer of a position as a member of the Rockefeller Institute followed and in 1922 he, Mrs. Landsteiner and his son, Ernest, now a surgeon in Providence, settled in New York.

He told Rous, who met him at the boat upon his arrival in the U.S.A. from The Hague, that he preferred to live in a small modest cottage with a rose garden by the sea—"such as I had in Schevenigen" and "it should cost no more than $50.00 a month." In a sense he never completely adjusted himself to life in an apartment house in New York City. Noises he abhorred and in anticipation of neighbors' complaints, he disposed of his piano which, incidentally, he played exceedingly well.

Landsteiner was a tall, erect, handsome man, stately and dignified in appearance who possessed an amazing vitality and stamina. His military bearing and his sternly flowing mustache failed to conceal an underlying but frequently suppressed spirit of warmth, gentleness and sincerity. To some he gave a first and deceptive impression of naiveté in worldly matters, but this was due to a pervading modesty and simplicity. He was utterly and completely dedicated to scientific endeavors and had a passion for clear, logical and concise thinking. His singlemindedness revealed itself when early in his career after obtaining his medical degree, he spent five years studying chemistry with Hantsch, Emil Fischer and Bamburger at a time when new vistas of bacteriology and serology were opened up following the pioneer work of Pasteur and Koch. Among his publications are five papers on organic chemistry and to these he occasionally referred with much pride and satisfaction. It was this ability to apply the chemical weapon which enabled him to give body and substance to a theory on antibody specificity.

He preferred skilled hand labor such as he experienced in Europe and deplored the lack of craftsmanship in the United States. At times he revealed a pessimistic mood when thwarted or depressed by events in the laboratory or budget difficulties. On one or more of these occasions, he expressed his disquietude by stating he should have been a banker! In public he was shy and endured an obvious discomfort when presiding at the weekly seminars at the Rockefeller Institute.

In the laboratory he was an active, enthusiastic and tireless worker. His assistants had no choice but to follow the pace set by the master. It was not difficult for a fellow worker to call on Landsteiner if he had a message to deliver in the form of a worthy scientific problem. On these occasions he offered suggestions and gave advice generously. Never satisfied with the report submitted by an assistant, the work was repeated and all essential experiments, especially those which might lead to publication, he carried out himself. Nothing was left to chance. Then came the final experiment, so designed as to constitute a table in the manuscript. When completed there was no room for doubt or uncertainties because instinctively he knew that "nature guards her secrets jealously." Landsteiner once told me that his journalistic father was known for his stern and austere style. This heritage reflects itself in Landsteiner's extreme degree of self-discipline, and his unusual power of concentration. Whether inherited or acquired, the divine spark he possessed, but certainly the proper genes played a fundamental role.

His standards were high and invariably he dealt exclusively with issues of fundamental import. His mind was a relaxed one and the scope of his thinking was so comprehensive as to take into account the most unexpected. His publications are a model for clarity and compactness. He avoided short staccato sentences and repetition of words pained him; his sentence structures though long and complex, contained a unified concept. Within a comparatively short time and with much zest, he had mastered the English language as he had previously learned the more complicated Dutch. He preferred the King's English to Americanese. Only when under stress or anger did he revert to his native German—the better to express himself with some choice expletives!

I should like to describe the circumstances and background for Landsteiner's discovery of isoagglutination and the human blood groups. In 1900 the fundamental basis of immunological methods was already established but it was in terms of bacterial antigens and antibodies,

toxins and antitoxins. The red blood cell did not emerge as an antigen until 1898 when Bordet produced hemolysins in animals injected with red blood cells of a different species. Here Bordet anticipated Landsteiner who informed me years ago that independently he had produced the same phenomenon. When Bordet's paper was published, Landsteiner, who had already submitted his manuscript, promptly withdrew it.

It was not surprising that the first observations dealing with agglutination of red cells were made with the blood of patients. It was most tempting to associate red cell isoagglutination—in mixtures of serum of patients with red blood cells of other patients or healthy individuals—with pathologic states and indeed, this was Landsteiner's first clue. Not satisfied with this observation, he took the next logical step and tested the serum of healthy individuals with red blood cells of other healthy individuals. The first findings on normal isoagglutination reaction was reported rather inauspiciously in a footnote to another paper dealing with an unrelated theme. A fuller description of three blood groups, O, A and B was given in Landsteiner's paper of 1901* in which he mentioned the importance of taking into account these blood differences for the successful outcome of transfusions. He distinctly predicted that transfusions would be safe if patient and donor belong in the same blood group. The fourth and rarest of the blood groups, AB, was discovered by Sturli who at Landsteiner's request extended his observations to a larger series in a study with Decastello.

Two other important findings were announced in that same year. One was the rediscovery of laws of heredity originally published in 1865 by Gregor Mendel. The other was the observation by Ehrlich and Morgenroth on the production of isohemolysins in goats injected with large quantities of hemolyzed blood of other goats. In contrast to the regular occurrence in a man of four well defined blood groups, numerous finer individual differences were shown for goat red cells and later for red cells of cattle and chickens. Apparently immune isoantibodies offer finer reagents for red cell differentiation than do normal isoantibodies.

Observations on blood groups and transfusion bore no immediate fruit. The time was not yet ripe. Transfusions could not be carried out because of technical difficulties. Until anti-coagulants were discovered in 1915, independently by Lewisohn (honored by your society in 1955) and Agote and Hustin, successful transfusions were not possible without the most refined and deft surgical skills required for anastomosis of donors' arteries with recipients' veins. In the U. S., Hektoen and Ottenberg independently urged in 1907 that transfusions could be made safe by the use of a compatibility test and Ottenberg received the credit for having first successfully applied a compatibility test. The late Dr. Ottenberg was honored by your association in 1954.

With Richter, Landsteiner in 1902-03 identified isoagglutinins in blood stains and suggested their practical application in forensic medicine. Landsteiner abandoned blood groups for more important exploits and did not resume the subject of his first love until he became a member of the Rockefeller Institute in 1922. In the interval of 1903-1922 the following important discoveries could be attributed to Landsteiner.

The pathogenesis of paroxysmal hemoglobinuria (with Donath), dark field illumination for demonstration of spirochetes, the viral nature of poliomyelitis, the use of alcoholic extracts in the Wasserman test, that remarkable work which demonstrated the chemical basis of serologic specificity, the cultivation of Rickettsia in tissue cultures (with Nigg), his theory of hypersensitiveness to drugs and methods for affecting sensitization (mainly with Chase) and his concept of the individuality of human blood. This by no means exhausts Landsteiner's fundamental observations and I cite the following example which deals with the specificity of red cell antigens. It is not generally known that Landsteiner made some of the earliest observations on plant agglutinins now termed lectins. He provided evidence that contrary to opinion prevailing at the time, the plant agglutinins were specific. Boyd in his stimulating 1960 Presidential Address of the American Association of Immunologists, stated that it was this observation described in Landsteiner's book "The Specificity of Serologic Reactions" which inspired him to investigate numerous plant extracts. Boyd's studies along with those of Renkonnen and others made available a large variety of reagents specific for O (H), A, B and more recently, M and N.

With regard to paroxysmal hemoglobinuria, Ehrlich had interpreted the discoloration occurring in the finger tourniquet test as due to a vital factor in the endothelium of the blood vessels. But with much glee and a merry twin-

*A translation of this appears elsewhere in this issue.—Ed.

kle in his eye, Landsteiner informed me how "he put one over" on Ehrlich when he demonstrated hemolysis in the test tube containing patient's fresh serum and red cells, first chilled and then incubated at body temperature to allow for the action of complement.

To return to human blood groups, important contributions were made by von Dungern and Hirszfeld who in 1910 described the subgroups of A and more significantly demonstrated the heredity of the A and B factors as due to two independent sets of genes. But the genetic theory which finally prevailed i.e., three allelic genes, was brilliantly elucidated by the mathematician Bernstein without carrying out any blood tests. Finally, in 1919 the Hirszfelds announced that the incidence of the four groups differed in various races. It was not a simple task for the Hirszfelds to find an editor who had sufficient faith in their rather unexpected and unorthodox findings, but their paper was finally published in the *Lancet*.

At the Rockefeller Institute Landsteiner resumed experiments and studies on human blood groups but, in addition to a new environment and language difficulties, was frustrated by lack of a continuous supply of material. His classical studies on A and B antigens in red blood cells of primates (with Miller) appeared in 1925. In one of these papers he described the method of preparing eluates from agglutinated red cells by heat—a technic so useful in current studies. With Witt, Landsteiner studied again the subgroups of A and confirmed an earlier observation that group O sera contain what we now know as a cross-reactive antibody which is believed to be important in the pathogenesis of ABO hemolytic disease.

My first studies with Landsteiner on cold agglutinins in human serum revealed numerous fine intragroup differences and a relationship of O and A_2. The initial observations on the occurrence of group A and group B substances in cells other than erythrocytes i.e. spermatozoa, were made by Landsteiner and myself in 1926. The findings were not announced until after Yamakami described the presence of A and B inhibiting substances in seminal plasma and in one specimen of saliva. We did not test the seminal plasma so that there was a possibility that the effects observed with the once washed sperm cells may have been due to residual plasma. More recently Celano and I have confirmed and extended these observations with thoroughly washed sperm cells from non-secretors as well as from secretors. (Incidentally none of the other blood factors studied such as Rh, M or N could be demonstrated in sperm cells.)

The first paper on M consisted of three brief paragraphs given in 11 lines of text and two tables.

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A New Agglutinable Factor Differentiating Individual Human Bloods.

K. LANDSTEINER AND PHILIP LEVINE.
From the Laboratories of the Rockefeller Institute for Medical Research.

By absorbing a number of anti-human blood immune sera from rabbits with the blood corpuscles of certain individuals regardless of the group, fluids were obtained from a few sera which give a sharp differentiation of individual human bloods within the common blood groups.

Among 116 individuals selected from the four blood groups the distribution of the agglutinable factor (which may be designated as M) was as shown in Table II.

This reaction is distinguished by its intensity from some others known to show individual differences within the groups, such as the reactions with cold agglutinins.

This is a preliminary report. (Reprinted from the PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 24: 600, 1927.)

The same year we published another brief paper consisting of one full page of text in which we described the following:

1. New factors N and P.
2. A racial difference in M between colored and white.
3. Identification of M in blood stains.
4. Heredity of M as a Mendelian dominant.
5. A_1 as a hereditary property.
6. Serological and genetic relationship of M and N.
7. The occurrence of M in 8 chimpanzees but not in 5 gibbons.
8. A higher incidence and stronger reactions in P in colored than in white bloods.
9. Normal rabbit and horse sera as a source of anti-P.

A fuller discussion of the factors M, N and P, their heredity, racial differences and other serologic details was given in a series of papers extending from 1928-1932. In considering the heredity of M and N as a genetic system, we excluded independent genes, close linkage and we also considered the existence of more than two alleles "interacting or modifying effects of factors determining hitherto unknown agglutinable structures." We stated that M and N

were not linked with A and B and as for A and B there was no linkage with sex. In our family studies on M and N, there were five instances of violations of the genetic theory proposed, but in each case the husband and not the mother was involved.

Neither Landsteiner nor I were versed in the already complicated subject of genetics but at Schermerhorn Hall at Columbia University, T. H. Morgan and his associates, Bridges, Sturtevant and Weinstein were locating genes in the chromosomes of Drosophila. We called on Morgan and his co-workers for advice, counsel and discussion of the several implications of our genetic studies on M and N. I do not recall after 32 years that Morgan and his colleagues took exception to our terminology of M and N as alleles. In the light of our present knowledge, genetic usage would dictate the use of M^a for M and M^b for N or of i^m and i^a respectively.

While dealing with terminologies, the members of the A.A.B.B. may be interested to learn why we selected the letters M and N for the new blood factors. In the first place it was important to employ letters sufficiently removed in the alphabet from A and B. Since "immune" antibodies were employed, "M" and "N" were chosen also because they appear in the word "immune." It was obvious that the letters I and O (Jansky I) must be excluded and letters in the alphabet following P reserved for blood factors to be discovered later, or so we thought.

In our papers prior to 1928, we still employed the Jansky numberings I, II, III and IV. A footnote to the paper in the Journal of Experimental Medicine on "Individual Differences of Human Blood" read as follows:

> "In the present paper the nomenclature adopted by the American Association of Immunologists is used" i.e., **O.A.B.** and AB.

The next factor to be discovered was termed P and in one of our papers, we refer to P_1, P_n and P_a as the antigen selected by three different reagents—the immune agglutinin produced in rabbits, normal human serum (extra agglutinin-1) and normal animal serum respectively.

Historically, it is of interest that we succeeded only once in producing a potent anti-P in rabbits. We injected the red cells of a colored individual "Charles Hunter". Numerous attempts to reproduce this effect, i.e., immune anti-P in rabbits by injection of red cells met with failure, but some consolation was derived from persistent efforts on the part of Landsteiner, Strutton and Chase in that other rabbits, injected with the same "Charles Hunter" blood produced another rather elusive antibody called the "anti-Hunter" factor. At the time this factor was suspected as belonging to the MNSs system and more recently this has been established.

At this point of our studies, only Schiff in Berlin and Thomsen in Copenhagen were active in this area of endeavor. While the iron was still hot, we felt it important that other workers confirm and extend our findings. On the occasion of my first trip to Europe in 1928, I delivered to Dr. F. Schiff in his Berlin laboratories one vial each of anti-M and anti-N and together we prepared the absorptions of the anti-M with my own N blood. This got him started with a panel of test cells. At about the same time, Wiener, then a medical student in Brooklyn, displayed great interest in blood groups and he was presented with a sufficient supply of reagents to initiate his career in this field.

In 1929, 72 different types of bloods could already be identified, if one included a serum described by Ottenberg and Johnson. In a study of "Isoagglutinin Reactions of Human Blood Other Than Those Defining the Blood Groups," in 1929 Landsteiner and I described numerous examples of anti-A_1, anti-A_2 and anti-P and five examples of atypical agglutinins which reacted on about 16 per cent of all bloods and there is reason to suspect that these corresponded to anti-Lewis (anti-Le^a) named by Mourant in 1946.

As far back as 1929 we had available a sufficient number of reagents to differentiate each of the nine individuals working in the laboratory. You will note that Landsteiner was in group O, type N, P negative and positive with the serum of Ottenberg and Johnson. I was and still am in group A, subgroup A_2, type N, P positive.

One obvious source for finding antibodies for new blood factors was the recipient of repeated transfusions but in 1928 these were hardly available in New York City or elsewhere. And so arrangements were made with Dr. M. L. Janes to transfuse six volunteers each with the same ABO compatible donor blood. Five of the recipients failed to produce antibodies, but the serum of the sixth recipient agglutinated at 37 C., 40 per cent of 112 bloods tested. After 32 years one hestitates to venture a guess as to the possible relationship of this blood antigen to any one of the numerous blood factors known at present.

Thwarted in our efforts to find new human isoagglutinins, we turned our attention to animal bloods. Individual differences in rabbit red cells were demonstrated by means of immune

TABLE 1.

	BLOOD								
	v.d. Sch.	Ldst.	Henry	Mock	Ph.L.	Hdy.	Bl.	Kr.	A.W.**
Group	O	O	A	O	A	A	A	O	O
Reaction for M†	+±	0	++±	++±	0	++±	++±	0	+±
Reaction for N†	+	++	0	+	++±	+	0	++±	+±
Serum 1219 AA^2 (Agglutinin a')	0	0	+	0	0	+	0	0	0
Serum A, no. 740	+	tr.	0	±	+	+	tr.	+±	0
Serum B, no. 2038	±	0	*	0	*	*	*	+	0
Serum B, Menz	0	+	*	0	*	*	*	0	±

** A.W. = Prof. Arthur Wormall now Professor of Biochemistry at Medical College, St. Bartholomew Hospital, London.

* Isoagglutination.
† See (32).

Landsteiner, K. and Levine, P. "On Isoagglutinin Reactions of Human Blood Other Than Those Defining the Blood Groups." J. Immunol. 17:23, 1929.

isoagglutinins and a number of differences in chicken red cells were revealed in tests with selected beef sera and sera containing antibodies of the Forssman variety. Perhaps the most significant of the studies on animal blood, at least from the evolutionary viewpoint, dealt with attempts to differentiate human and chimpanzee red cells and serum proteins by immunization of chimpanzees with human red cells and human serum. More refined methods of differentiating human and chimpanzee red cells of the same ABO groups were described, as well as the occurrence of individual differences in chimpanzee red cells. One out of three chimpanzees injected with human serum produced weak but distinct precipitins for human serum, thus providing evidence for fine differences in their serum proteins. Such differentiation could not be made in tests with anti-human or anti-chimpanzee precipitins produced in rabbits. I cannot leave the memory of these experiments without recalling the number of rather thrilling adventures in injecting the chimpanzees who displayed some very human talents in projecting their saliva with remarkable accuracy—their secretor status was of little interest to us at that time—or by withdrawing violently the loaded syringe and needle from the site of the injection—at a respectably safe vantage point—the spectator could have gained the impression that it was the chimpanzee's intent to immunize either me or Paul, the animal keeper!

In 1930 the announcement was made that Landsteiner was to receive the Nobel Award for his discovery of the blood groups and their role in transfusions. At the time he informed me that he would have preferred to have been honored for his contributions on the chemical basis for serologic specificity—a monumental work which substituted fact for Ehrlich's symbolic house of cards. Be that as it may, when Landsteiner was requested by the Nobel Committee to submit the names of future recipients, his choice was T. H. Morgan who was named as Nobelist in 1932. I felt deeply honored in my humble role in helping Landsteiner frame his supporting statement.

After leaving Landsteiner in 1932, we had finally agreed that I was to embark on new vistas rather than to continue with studies on blood groups. To abandon the study of antigens in human red cells and to reverse the momentum of my intense interest in this area, was not a simple decision for me to make. The line of least resistance was to continue the studies on individual differences of human blood. After three years at the University of Wisconsin as a member of the medical faculty, I resumed blood studies at the Newark Beth Israel Hospital. It was my good fortune to have referred to me by Dr. Rufus Stetson in October 1937, the blood specimen of a patient suffering from a transfusion accident at the very first transfusion following a stillbirth. The patient's name was Mary Seno and she is identified in the paper as M.S. From this paper I quote as follows:

> "In view of the fact that this patient harbored a dead fetus for a period of several months, one may assume that the products of the disintegrating fetus were responsible not only for the toxic symptoms of the patient but also for the isoimmunization. Presumably the immunizing property in the blood and/or tissues of the fetus must have been inherited from the father. Since this domi-

nant property was not present in the mother, specific immunization conceivably could occur."

"Agglutinins of this sort can rarely be investigated thoroughly because of their tendency to diminish in activity and eventually to disappear. Consequently attempts were made to produce a hetero-immune agglutinin of identical or similar specificity by repeated injections of sensitive blood into a series of rabbits. These experiments met with failure, since suitable absorption tests with such serums failed to reveal the presence of the desired agglutinin."

The paper was not submitted for publication until after the immune character of the agglutinin—activity at 37 C. and its temporary nature—was established. The agglutinin, of course, of the saline variety diminished in activity at two months and disappeared entirely at the end of one year.

During the years 1937-1939 while I was studying the blood of Mary Seno, Landsteiner and Wiener had demonstrated an M-like factor in the red cells of rhesus monkeys as revealed by the production in rabbits of anti-M like antibodies following the injection of rhesus red cells. Further studies revealed that some of the rabbits produced another antibody reacting with about 85 per cent human red cells. To this the name Rh was assigned. This important discovery was announced by Landsteiner and Wiener in 1940 when Wiener and Peters found that antibodies of the same or related specificity could be produced in man following repeated blood transfusion.

My studies carried out in 1939-1940 showed that the same antibody was produced as a result of transplacental isoimmunization of Rh negative women. These patients were referred to me because of hemolytic transfusion accidents at the very first transfusion. Since these patients had stillbirths with and without hydrops, or infants with frank erythroblastosis fetalis, it was not a difficult matter to suggest that the source of the hemolytic process in the infant—until then an unsolved mystery—was the action of the maternal anti-Rh on the susceptible Rh positive affected infant.

After the relationship to hemolytic disease of the newborn was established, large quantities of the several varieties of human anti-Rh sera became available and the use of the animal anti-Rh sera was abandoned. In 1943 the blocking or the incomplete antibody was described independently by Diamond and Denton, Wiener and Race. With the discovery by Coombs *et al* of the antiglobulin reaction and the introduction by Morton and Pickles of enzyme treated red cells, a battery of methods were then available for the discovery of antibodies specific for many new red cell antigens.

In early studies only saline agglutinins were observed so that the main body of evidence for the role of the Rh factor in hemolytic disease of the newborn depended upon a disproportion of Rh negative mothers with any one of three saline agglutinating sera giving respectively—87, 85 and 70 per cent positive reactions. The greatest discrepancy was found with the 85 per cent serum which was and still is considered as standard. Absorption tests showed that the 87 per cent serum consisted of what we now know to be anti-Rh_0 (anti-D) plus anti-C (rh′). But in these 1941 studies the clear-cut separation of the two antibodies was obscured by what is known today as anti-G of Allen.

In 1941 we also observed that our anti-Rh_0 (anti-D) serum gave fewer negative reactions and a greater number of weakly positive reactions when tested with the blood of Negroids. These observations were never published.

The first anti-Hr agglutinin was found in a serum of an Rh positive mother (K.F.) of an affected infant. This serum gave strong agglutination reactions on all bloods which failed to react with our anti-C (rh′). Although the reciprocal relationship was not complete, especially with C positive bloods, it seemed that we were dealing with contrasting reactions which signified a genetic relationship in what we now know as the C-c system and hence, I reversed the letters in Rh and for better or worse, the term Hr was born.

After the description of the E (rh″) antigen in 1943 and especially the predicted contrasting antigen e (hr″) in 1946, the way was paved for the alignment of the proponents of the two contrasting genetic theories, linkage or multiple alleles. One of Landsteiner's last papers dealing with the already complex heredity of the Rh types appeared in 1943 in a paper with Wiener.

At this point, I should like to discuss briefly the impact of Landsteiner's 1900 discovery as viewed from 1960 and beyond. From 1943 to date, we have travelled fast toward establishing the concept of individuality of human red cells by virtue of varying combinations of an ever increasing number of antigenic components.

Including ABH secretions, there are now at least twelve genetically independent antigenic

systems and this does not include a number of low incidence and several high incidence blood factors. While the ABO system does not contribute much toward individuality, the Rh-Hr system now contains at least 17 antigenic components. At least 14 different antigenic determinants are known in the once simple MNSs system. To complicate the situation still further, additional factors have been discovered in the short interval required for the completion of this manuscript. The complexity of the P system is just emerging and the end for these and other genetic systems is not yet in sight. This clear-cut and almost perfect individuality of the red blood cell as we know it, would not have been surprising to Landsteiner.

In a broad sense blood groups or serologic differences in the elements of the blood should include leukocytes, platelets and proteins such as, Gm. groups, haptoglobins, transferrins and hemoglobins—only the latter thus far associated with macromolecular disease. The major antigenic components found in leukocytes and platelets appear to differ from antigens encountered in red blood cells.

Landsteiner had searched for individual differences in serum proteins but failed to demonstrate them with the methods available to him. In our paper of 1926 we wrote as follows:

> "In fact while it was easy in several cases to obtain hemolytic isoantibodies, the possibility of producing isoantibodies to proteins has not been demonstrated with certainty, a dissimilarity hitherto unexplained."

Ultimately the antigenic differences in the red cells are a reflection of finer chemical differences which are under genetic control. Landsteiner and others had studied A, B and O activity in animal sources and human salivas and by their chemical analysis these could not be differentiated. Using his method of inhibition of serologic reactivity by haptens, Morgan and Kabat had shown that the stamp of ABO and Lewis specificity in the form of terminal sugar groupings is imprinted, probably in a series of steps on a basic mucopolysaccharide. A block in the stepwise genetic control in the synthesis of Lewis substances, ABH secretion and expression of ABO on the red cell is illustrated in Ceppellini's interpretation of the findings of the Bombay type of blood in the family which I studied in 1955.

In the absence of water soluble substances, biochemical studies on the numerous antigenic components of red blood cells other than A, B, Lewis and P are fraught with difficulties. However, preliminary information may be obtained in studies to determine the simple substances which may inhibit the antibodies for the numerous antigenic determinants. As a model one may cite the recent observations of Hackel and Boyd that certain nucleic acid derivatives and "unnatural" sugars inhibit the action of Rh antibodies. Landsteiner's great contribution was his concept—developed very early in his career—that any serologic difference, be it agglutination of red cells or bacteria or precipitation of soluble substances, points to a difference in chemical groupings. He had stated that red cells "contain different substances which, however, *for the time being* are distinguishable only by serologic methods."*

Today, on this anniversary, it has been our privilege to review the pioneering work and contributions of Karl Landsteiner in the field of blood groups. Through the annual Landsteiner Award, the A.A.B.B. commemorates the memory of a dedicated man who gave dignity to every problem he chose to undertake. To him the printed word had a unique significance and sanctity. One did not have to be long in his presence to discover that here, indeed, is an inspired man who in his long and brilliant career always "aimed at infallibility without ever claiming it."

Finally, I should like to quote from Sir Oliver Lodge's translation of Richet's 1923 delightful book, "The Natural History of a Savant," a remark which characterized Landsteiner as a genius.

> "No one has the right to encumber Science with premature assertions."

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* Quotation and underscoring taken from obituary notice by Rous referred to previously.

(A complete list of Landsteiner's publications may be found in the Obituary Notice published in the *Journal of Immunology*, 48: 1, 1944—Ed.)

Book Reviews and Abstracts

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Reviews

The Anti-Globulin (Coombs) Test in Laboratory Practice. I. Dunsford and J. Grant. Springfield, Illinois, Charles C Thomas. 1960, 120 pp.

IT IS RARE that a laboratory procedure becomes the subject of a monograph published in book form. The Coombs or anti-globulin test has proved to be a valuable tool in both routine and investigative blood group work. It is being used on many problems that concern antibodies of one sort or another. As time goes on it will undoubtedly be used or at least tried in a much wider circle of problems.

The monograph by Dunsford and Grant has been designed primarily for workers in blood banking and blood group serology. Following a brief discussion of antigens, antibodies and the basic mechanism of the test, there follow instructions for animal inoculations, preparation and standardization of serum and finally technics, causes of false reactions and applications of the test itself.

The reviewer notes that the order of incidence of causative antibodies in hemolytic disease implied on page 87 is not in keeping with the experience of others. A purist would question the use of *congenital* and *hereditary* as synonyms. The hour and a half incubation period recommended in the anti-globulin compatibility test seems a rather drastic requirement. Clinicians quite understandably become peevish at the delay. The tile technic employing 50 per cent suspensions of erythrocytes is not frequently used in this country but has advantages. These minor criticisms in no way detract from the value of the presentation.

The material is well organized and presented in a lucid, readable manner. The references are well selected and typographical errors are few.

Some may feel that this monograph is too brief; however, it is in keeping with the intention of the authors to avoid an exhaustive review and still furnish a broad basis on which the student can build. It is highly recommended to all who are responsible for performing the anti-globulin test and want to understand it better. —*J. B. Ross.*

Cellular Aspects of Immunity—A Ciba Foundation Symposium. London, J. A. Churchill, Ltd., 60 shillings (approx. $8.50).

THIS VOLUME is a record of the scientific proceedings of a conference held at the Cercle Culturel de Royaumont in 1959, under the sponsorship of the Ciba Foundation. The participants were 34 internationally known immunologists; the volume includes both the papers presented and the valuable discussions that followed. The symposium covers every fundamental aspect of the central theme of immunology which at present seems to be the immunologically competent cell, rather than the antibody. Chapters range from the electron-

microscopic ultrastructure of immunologically competent cells, to the in vitro action of antigen-antibody complexes on thrombocytes and erythrocytes, and to the multiple autoantibodies to cell constituents in systemic lupus erythematosus. Of special interest is the panel discussion on the clonal selection theory with Burnet, Lederberg, Good, Grabar, Medawar, Monod, Simonsen and Stertzle as active participants.

Also included are studies on the plasma cell and antibody formation, on antibodies in homograft reactions, on the cellular origins of the serum proteins involved in immunity and on various other aspects of immunological reactions. The informality of presentation of the data and the critical evaluation in the published discussions make this an unusually readable and highly informative review.—*H. Fudenberg.*

Abstracts

Blood Groups and Antibodies

The I antigen and antibody. W. J. Jenkins, W. L. Marsh, J. Noades, P. Tippett, R. Sanger and R. R. Race. National Blood Transfusion Service, Brentwood, Essex, England. Vox Sang. 7: 97, 1960.

THE ANTIBODY in the serum of each of eight available cases of cold agglutinin acquired hemolytic anemia was identified as anti-I. The antibody present in normal sera and previously called "non-specific cold agglutinin" was also identified as anti-I. The antigen I was found to be but feebly developed at birth, so that newborn red cells can probably serve as an I-negative control cell in laboratories lacking this rare type.—*H. F.*

Further studies of the I antigen and antibody. Patricia Tippett, J. Noades, R. Sanger, R. R. Race, L. Sausais, C. A. Holman and R. J. Buttimer. Vox Sanguinis 5: 107, 1960.

THIS PAPER is devoted to the study of five people with the phenotype i and whose serum contains anti-I activity. Each had varying degrees of lack of I. None of the antisera agglutinated the untreated cells of Mr. M. These anti-I antibodies all failed to react with cord cells. The natural anti-I serum of Mr. M. and the auto anti-I sera do react with cord cells but much more weakly than with adult cells. The saliva of secretors and non-secretors failed to inhibit the anti-I activity in any of the sera. The studies revealed that the I antigen strength present in the Puerto Rican and the four Negroes studied was higher than in the cells of Mr. M. The authors likened these results to the ABO system in that Mr. M. would correspond to an O person with anti-A and anti-A_1 in his serum and the others would correspond to an A_2 person with anti-A_1. The possibility of a relationship between I and the ABO system is pointed out because i cells react much less strongly with anti-H and anti-O than do the cells of normal people and group O cells are on the average slightly but definitely more strongly agglutinated than other cells of other groups by the auto anti-I sera. Other features which give slight indications of this relationship are mentioned. It was also found that the I antigen is but weakly developed at birth. i cells have to be distinguished from the so-called Bombay type and from individuals who have anti-H or anti-O in their sera. Anti-I does agglutinate "Bombay" cells while anti-H and anti-O do not. Anti-H and anti-O can be distinguished from anti-I in that they agglutinate O cells more strongly than A_1 cells and secretor saliva inhibits anti-H but not anti-I.—*T. J. G.*

Antibody of blood-group specificity in simple ("cold") haemolytic anaemias. W. Weiner, N. K. Shinton and I. R. Gray. Blood Transfusion Service, Edgbaston, Birmingham, England. J. Clin. Path. 13: 232, 1960.

ANTI-I WAS the name given by Wiener *et al.* (1956) to the antibody in the serum of a patient with acquired haemolytic anaemia of the "cold" antibody type. Only five I negative, i.e. *ii* persons, were found in 22,000 samples tested. The present study demonstrates that two patients with acquired haemolytic anaemia of the cold agglutinin variety have the antigen I on their red cells and anti-I in their serum. The demonstration depends on distinguishing between *ii* and I blocked by cold incomplete anti-I as well as by serologic reactions using eluates prepared from I positive and I negative red cells. The presence of anti-I in a truly homozygous *ii* patient is compatible with health. —*H. F.*

The inheritable B-like character in persons of blood group A. J. Andersen. Statens Serum-institut Blood Grouping Department, Copenhagen. Blood 16: 1163, 1960.

THE AUTHOR previously reported an individual who appeared to be of group AB but had anti-B in his serum. The B character was

weaker than normal. Several sera which gave distinctly positive reactions with these cells were selected for testing 50,000 blood samples. Five further examples were thus found and two additional ones were found later. All eight samples belong to group A_1. Two were selected for special study because families were available. In one family Pe the propositus had two sons and a grandson with the weakly reacting B factor. The propositus tested as group A_1 whereas the three other members of the family with the unusual reactions were group A_2. Inheritance could not be demonstrated in the second family studied. Individuals with the unusual blood were labeled A_1^b and A_2^b. The A_1^b cells reacted mainly with anti-B antibodies from A_2 individuals whereas the A_2^b cells reacted with most immune anti-B sera but more strongly with those from A_2 individuals giving a titer only one tube less than regular B cells. Absorption of sera giving positive reactions with normal B cells removes the agglutinin which reacts with the A^b cells and the eluate from the B cells does not react with the A^b cells. Absorption with A^b cells removes only the reactivity with A^b cells. The eluate from A^b cells reacts at 4 C. with A_1, A^b and B cells but not with A_2 or O. This property seems to be a stable attribute of the cells in most of these individuals but in one, Do, studies performed five years later indicated that it had been a transient phenomenon. The possible modes of inheritance of this blood group are discussed.—*T. J. G.*

Family blood group and secretion studies. C. McNeil, E. F. Trentleman, W. M. Helmick, R. Orlob and J. G. Haskell. Holy Cross Hospital Research Foundation, Salt Lake City, Utah. Vox Sanguinis 5: 164, 1960.

USING quantitative saliva secretion studies it was shown that individuals of blood groups A_1, A_1B and most A_2 individuals secrete salivary substances which neutralize the ability of anti-A serum to agglutinate both A_1 and A_2 cells. An occasional A_2 and almost all A_2B people failed to secrete the A_1 neutralizing factor. Only one A_2B individual who secreted A_1 has been found and his saliva gave an A_1 neutralization titer of only 1:64. This reduction in A_1 salivary secretion by A_2B individuals suggests that the B gene may suppress the A_1 activity when in combination with the A_2 gene. The level of secretion of B substance in the saliva is much more consistent.—*T. J. G.*

Rh_0 (D) genotype and red cell Rh_0 (D) antigen content. S. P. Masouredis. University of California Medical Center, San Francisco, California. Science 131: 1442, 1960.

AN ELEGANT technic involving elution of I^{131}-labelled anti-Rh_0 antibody from Rh_0 positive red cells, has been used to measure (quantitatively) red cell antibody uptake. Studies with red cells obtained from 199 Rh_0 donors revealed a bimodal distribution of I^{131} uptake. Family studies revealed cells with the lower antigen content, as measured by this method, to represent heterozygotes. Homozygous Rh_0-positive red cells appeared to bind 1.6 times as much antigen as heterozygous Rh_0-positive red cells. This difference presumably represents the difference in the number of antigenic sites on the red cells.—*H. F.*

Anti-A Agglutinins from the Seeds of Hyptis suaveolens Poit. G. W. G. Bird. Armed Forces Medical College, Poona, India. Brit. J. Haemat. 6: 151-153 (Apr.) 1960.

A NEW seed extract with blood group agglutination activity has been found. This plant grows wild in India; it is of a different family from those heretofore tested. This seed extract has anti-A activity although it is unable to detect most examples of A_2 in A_2B cells.—*J. B. R.*

Acquisition of blood-group B-like bacterial antigens by human A and O erythrocytes. G. F. Springer and N. J. A. Hahn. University of Pennsylvania, Philadelphia. 8th Proc. Internatl. Soc. of Blood Transfusion, Tokyo 1960.

SOLUBLE blood-group B active materials which coat human erythrocytes of all age groups making them agglutinable by anti-B antibodies have been found by the authors in culture media in the declining phase of growth of *S. berkeley* O_{43}, *Arizona* O_{21}, *E. coli* O_4, *E. coli* O_8, *E. coli* O_{55}, *Proteus mirabilis*, organisms of Citrobacter, Klebsiella, Bethesda-Ballerup, pasteurella pseudotuberculosis and intermediate coliform groups and strains of *E. coli* O_{86}:B7, especially after heating at pH 7.3. There is no apparent influence on agglutinability of pre-existing blood-group receptors but coating renders the erythrocytes polyagglutinable.

Of numerous different blood-group agglutinins which were adsorbed on and eluted from various human erythrocytes only eluted anti-B antibodies agglutinated O erythrocytes after they had been coated with *E. coli* O_{86}. Furthermore, only anti-B antibodies could be adsorbed

on and eluted from *E. coli* O_{86} coated human O erythrocytes. This indicates that of the blood-group agglutinins, only anti-B is taken up by O erythrocytes coated with *E. coli* O_{86} polysaccharides. The eluted anti-B agglutinins are inhibited by small amounts of blood-group B active substances of mammalian and bacterial origin but not by A and H(O) active substances.—*G. F. S.*

Blood group specific decomposing enzymes from bacteria. S. Iseki. Gunma University, Maebashi, Japan. 8th Proc. Internatl. Soc. of Blood Transfusion, Tokyo 1960.

O(H)-decomposing enzyme from *Bac. fulminans* decomposes water-soluble as well as red cell O(H) substance, converting them into heterophile human red cell antigen of *Shigella dysenteriae*. A-decomposing enzyme from *Cl. tertium* decomposes water-soluble as well as red cell A substances, converting them into O(H) substances. B-decomposing enzyme from *Cl. maebashi* decomposes water-soluble as well as red cell B substance, converting them into O(H) substances. B-decomposing enzyme liberates from O(H) substance about 30 per cent of the original fucose, and about 2 per cent of the original N-acetylhexosamine and galactose, and from A or B specific substance about 30 per cent of the fucose, about 10 per cent of the galactose, and about 2 per cent of the N-acetylhexosamine. A-decomposing enzyme liberates from A specific substance about 20 per cent of the galactose and about 8 per cent of the N-acetylhexosamine. B-decomposing enzyme liberates from B specific substance about 8 per cent of the original galactose, and liberates no sugar from O(H) and A substances.—*G. F. S.*

Storage and Transfusion

Clinical use of glycerolized frozen blood. L. L. Haynes, J. L. Tullis, H. M. Pyle, M. T. Sproul, S. Wallach and W. C. Turville. U. S. Naval Hosp., Chelsea, Mass. J.A.M.A. 173: 1657, 1960.

THIS REPORT demonstrates that red blood cells can be glycerolized, stored in the frozen state for two years or more, deglycerolized and transfused into patients on a large-scale practical basis. Use of a special centrifuge (Cohn fractionator) makes the processing steps rapid while maintaining sterility.

An average of 18 per cent of donor cells was lost during processing, but the remaining cells had normal viability on transfusion. Only four minor non-specific transfusion reactions occurred in a series of 1,014 transfusions in which the red cells were resuspended in 5 per cent albumin. This low reaction rate suggests that most of the factors causative of transfusion reactions were lost during the washing process.

Red cells resuspended in albumin were effective in maintaining patient red cell volume even with massive replacement. No bleeding tendency was encountered in five cases who received 16 units of red cells in albumin. When desired, donor plasma was frozen in a separate container attached to the red cells and used to resuspend them after removal of the glycerol. Heparinized plasma was stored as long as five months without loss of heparin activity and was then successfully added to red cells used in a pump-oxygenator for open heart surgery.

The processing cost per unit of blood was estimated at $16.40, including amortization of the basic equipment. Two Cohn fractionators were able to glycerolize or deglycerolize 400 units of blood per month.

This technic is particularly valuable for the storage of rare types of blood.

[More data are needed before one can accept the implication that massive exchange transfusions of red cells suspended in albumin (free of platelets and coagulation factors) fail to induce a hemorrhagic state known to occur with massive transfusions of stored bank blood.—Ed.] —*H. A. P.*

Observations on the effect of purine nucleosides on red-cell preservation. P. L. Mollison and M. A. Robinson. Post Graduate Medical School, London W. 12, England. Brit. J. Haematology 5: 331, 1959.

A GREAT variety of experiments were performed in which adenosine and inosine were added at the beginning of storage, at weekly intervals, or after a prolonged period of storage in ACD and with incubation for one hour at 37 C. in some instances before doing survival studies. In some preliminary experiments it was observed that the restoration of 2,3-diphosphoglycerate (2,3-DPG) in stored cells was significantly greater when the cells were incubated with purine nucleoside and thiamine than when they were incubated with the nucleoside alone. In the present study this was not confirmed. Inosine does not seem to exert as favorable an effect as adenosine because the ATP level is not as high and neither is the 2,3-DPG level after addition of inosine. The survival of the red cells was not as good either. No definite quantitative correlation could actually be shown between ATP levels and survival

although there is some suggestion. Gabrio et al. believed that inosine was more logical because there was no adenosine phosphorylase whereas a phosphorylase capable of acting on inosine and guanosine has been isolated from red cells.

Present results indicate that when red cells have been stored their post-transfusion survival is not improved by incubating them at 37 C. for one hour before re-injection. The beneficial effect of adenosine on viability was confirmed whether adenosine was added at the beginning or at the end of the storage period or at intervals during the storage period. Post-transfusion survival fell to 50 per cent after about 60 days whereas in ACD the 50 per cent survival point was reached in 30 to 35 days.—*T. J. G.*

Storage of red cells for blood-grouping after freezing in liquid nitrogen. R. G. Huntsman, B. A. L. Hurn and H. Lehmann. Guy's and St. Bartholomew's Hospitals, London, England. Brit. Med. J. 2: 118, 1960.

SMALL SAMPLES of red cells from blood collected in E.D.T.A. were preserved for blood-grouping studies by freezing in liquid nitrogen. Prior to freezing cells were prepared by adding two volumes of packed cells to one volume of 40 per cent sugar solution. Although both sucrose and dextrose solutions were satisfactory, dextrose offered some advantages. Cell suspensions were dropped slowly into tubes of liquid nitrogen by means of pasteur pipettes. Freezing was instantaneous. The final preparation consisted of small globules which could be handled with forceps. The frozen droplets were thawed by pouring them into saline at 45 C. When cells were tested for A, B, C, D, E, c, M, N, K, and Fy no loss of reactivity was demonstrated.—*L. R. G.*

Open-heart surgery without the need for donor-blood priming in the pump oxygenator. W. B. Neptune, J. A. Bougas and F. G. Panico. New England Deaconess Hospital, Boston, Massachusetts. New Eng. J. Med. 263: 111, 1960.

AN ARTIFICIAL heart-lung machine that does not require the use of heparinized donor blood has been developed by the authors. The oxygenator is of small capacity and is primed with 500 to 1,000 ml. of saline solution.

The design permits circulation of blood in a layer below much of the saline which thus never enters the patient. Ordinary ACD bank blood is given intravenously to replace patient blood lost during and following surgery. In a selected series of 25 cases, two to nine units were required. The usual amount needed is four units. Fifteen per cent of cases developed a delayed anemia seven to ten days after operation, requiring further transfusion.

Fairly rapid flow rates of 70 to 90 ml. per kilogram of body weight were achieved with mean aortic blood pressures of 70 to 90 mm. Hg. Changes in plasma hemoglobin, blood platelets, fibrinogen and various other blood components followed the same course as those usually observed with other pump-oxygenators.

[The results reported are impressive, and the elimination of heparinized donor blood would relieve blood banks of a tremendous task. However, it should be emphasized that much additional work is required before this oxygenator can be used with a degree of safety equal to that of the machines in more general use. Adequate oxygenation of blood in a device so small can be achieved only by bubbling oxygen through the blood. There is much evidence that small high-flow bubble oxygenators carry a real risk of sending gas emboli into the patient and, perhaps even more important, emboli of the antifoam agent which must be used to convert the bubbles to liquid blood. In addition, the extra amount of saline infused into the patient, added to the ACD solution which forms one-fifth of every pint of citrated blood, may provide a load of water and sodium more than some damaged hearts can tolerate.—Ed.] —*H. A. P.*

A critical assessment of the use of blood transfusions during major gastric operations. B. J. Wilson and K. O. Adwan. U. of Texas Southwestern Med. School, Dallas, A.M.A. Arch. Surg. 80: 760 (May) 1960.

Overtransfusion in the operating room. C. J. Costello, Barnes Hospital, St. Louis, *Ibid.* 80: 843 (May) 1960.

WILSON AND ADWAN reviewed 100 consecutive patients who had elective subtotal gastric resections; only two were given blood transfusions during operation: 1. there were no deaths; 2. convalescence was not prolonged; 3. the incidence of complications was not increased; 4. transfusion complications including circulatory overload were avoided; 5. the incidence of hypotension during operation was not increased. In 44 patients the hemoglobin was less than 12 Gm. per cent, in 12 less than 10 Gm. per cent prior to operation. Balanced salt solutions were used to treat loss of blood volume during operation. In animal experiments, the authors re-

port that dogs transfused prior to a standard hemorrhage, survived less readily than untransfused dogs.

Costello described two operative cases who were given too much blood: one died and the other required a therapeutic phlebotomy. Shock, occurring in the operating room, may paradoxically be due not to deficiency but to excess of blood. Bleeding from a surgical wound may result from circulatory overload.—*J. B. R.*

A report on 500 cases of bleeding peptic ulcer treated medically with minimal transfusion of blood. W. K. Manning and K. O'Connor. Repatriation General Hospital, Concord, New South Wales, Australia. Med. J. Australia. 1: 918, 1960.

After surveying 250 cases of bleeding peptic ulcer, it was concluded, among other things, that hemorrhage tends to be self-limiting and the free administration of blood transfusions tends to increase the likelihood of further hemorrhage.

A series of 500 additional cases was then treated. Transfusion was withheld entirely whenever possible and in all cases as long as possible. Transfusion was used only for the relief of anoxia, local or general. It was seldom considered if the hemoglobin value was known to be 4 Gm. per cent or more. Only 13 patients received blood transfusion as an emergency measure. The largest amount transfused was 3,500 ml. Treatment largely consisted of assignment to a special ward, immediate sedation and frequent feeding.

A mortality rate of between 1 and 2 per cent was achieved. Mortality was closely correlated to the existence of serious co-existing disease. —*J. B. R.*

A clinical survey of blood transfusion. C. W. Graham-Stewart. St. Thomas's Hospital, London, S.E. 1, England. Lancet 2: 421, 1960.

Frequent shortages of blood in a London hospital prompted the author to survey the use of blood in an attempt to learn whether the supply should be increased or the use made more discriminating. The hospital staff was forewarned that the survey would be made and, during it, there were 16 per cent fewer transfusions than expected. Nevertheless 6.5 per cent of the transfusions were given unnecessarily. The authors close with eight rules that may be expected to reduce the number of unnecessary transfusions.—*J. B. R.*

Continuing hazard of air embolism during pressure transfusions. McL. Ruesch, S. Miyatake and C. M. Ballinger. University of Utah, Salt Lake City, Utah. J.A.M.A. 172: 1476, 1960.

In a period of four years, the authors noted four occurrences of pulmonary air embolism with three deaths in their community. Each episode occurred when air was blown under pressure into a blood bottle to ensure rapid delivery of blood.

Three technics available to transfuse large amounts of blood rapidly without incurring the danger of air embolism are described. A commercially prepared in-line pump can administer a unit of blood in three to five minutes but it requires an extra person to keep the blood running fast. A glass float, which descends to block the outlet when the last of the blood is gone, permits the safe use of air under pressure in the blood bottle, but it is expensive and must be cleaned and reassembled after each use. A plastic bag, which may be squeezed by hand or with a pressure jacket, will infuse 500 ml. of blood at a steady rate in two to five minutes. Other advantages of plastic bags include convenience in storing and shipping, ease of disposal of used bags and little breakage. Disadvantages include a longer period for collection of blood from donors and need to use a larger needle as well as greater expense.

The authors conclude with a plea for the universal use of plastic donor blood bags.

[In their comparisons between plastic bags and bottles, the authors make several incorrect or misleading statements. They state that red cell and platelet viability is better in blood collected in plastic bags than in siliconed bottles. Current evidence suggests that there is little difference between the two. They state that a smaller volume of anticoagulant is required in a plastic bag. This is not true. Finally they fail to point out that the longer donor bleeding time and use of a larger needle is a requirement of a gravity collection as opposed to vacuum and has nothing to do with the container in which the blood is collected.—Ed.]—*H. A. P.*

Use of air pressure to speed up blood transfusion. Editorial. J.A.M.A. 173: 1032, 1960.

In 1953 the Committee on Medicolegal Problems pointed out that blood transfusion under air pressure is to be condemned, but it would appear that it is still in common use throughout the country. Air embolism as the cause of a patient's death after a blood transfusion may

be considered prima facie evidence of negligence. The time has come to prohibit the use of this method of transfusion by appropriate laws in our Sanitary Codes.

When massive amounts of citrated blood are transfused, 10 ml. of 10 per cent calcium gluconate should be injected intravenously for every 1,000 ml. of blood.—*H. A. P.*

A case of malignant tertian (plasmodium falciparum) malaria after blood transfusion. D. B. Grant. M. S. Perinpanayagam, P. G. Shute and R. A. Zeitlin. Brook General Hospital, London, S.E. 18, England. Lancet 2: 469, 1960.

FOLLOWING receipt of three units of whole blood, an English woman developed malaria due to plasmodium falciparum. One of her donors was a native of Nigeria in whose blood similar parasites were found, but only after intensive search. He had never had clinical malaria. The donor blood had been refrigerated at 4-6 C. for 14 days prior to administration. This case illustrates the possibility of transmitting malaria from any donor who has resided in a malarious area.—*J. B. R.*

Hemorrhage and transfusion as the major cause of cardiac arrest. H. H. LeVeen, H. S. Pasternack, I. Lustrin, R. B. Shapiro, E. Becker and A. E. Helft. New York Medical Center, New York, N. Y. J.A.M.A. 173: 770, 1960.

ANALYSIS OF 157 cases of cardiac arrest led to the conclusion that transfusion of blood containing high levels of plasma potassium was primarily responsible for cardiac arrest in 50 cases and played a contributory role in 18 more. In the group in which transfusion had been the primary cause, rapid administration of large volumes of blood was common. Nineteen cases had received over 500 ml. of blood in less than 15 minutes. All had received more than 2,000 ml. during operation, 43 had received more than 3,000 ml., and 31 had received more than 4,500 ml. The average storage age of the blood was 11 days, and the authors' figures indicate that their bank blood had a plasma potassium level at the time that corresponds to the range reached in 21 days in most previously reported series. They believe this is due to the mechanical trauma caused by collection of blood elsewhere before transportation to their hospital. The average total dose of plasma potassium administered to the cases in which transfusion was the primary cause of cardiac arrest was 77.7 mEq.

The ability to tolerate large amounts of infused potassium is decreased by hemorrhagic hypotension, and the authors make a plea for replacement of blood as it is lost to avoid both a drop in blood pressure and the need for rapid massive blood transfusion.—*H. A. P.*

Transfused blood and cardiac arrest. Editorial. Lancet 2: 249, 1960.

IN THEIR recent article LeVeen et al. (J.A.M.A. 173: 770, 1960) point out that the chances of inducing cardiac arrest by massive transfusion are greater the longer the blood has been stored (due to higher plasma potassium levels). Anoxia, hypercapnia and shock should be avoided. Intra-arterial transfusion will cause more potassium to be removed by the peripheral tissues before the blood reaches the heart than if the blood is injected into a vein.

The use of norepinephrine rather than epinephrine is advised, since the latter releases potassium from the liver. Blood should be warmed to room temperature before administration. Monitoring venous pressure and electrocardiographic changes may provide warning of impending cardiac arrest.—*H. A. P.*

The use of a special preservation medium for the maintenance of platelet viability at 4 C. M. Baldini, S. Ebbe and W. Dameshek. New Eng. Center Hosp., Boston, Mass. Blood 15: 909, 1960.

THE POST TRANSFUSION survival of rabbit platelets was measured after varying periods of storage at 2 to 4 C. in different media. Viability of stored platelets was expressed by an index that took into account both the maximum level achieved and their survival in the circulation. For comparison, the viability of fresh platelets was expressed as an index of 100 per cent.

Platelets stored for 24 hours in 0.02 per cent Triton in normal saline had a viability index of only 2 per cent. Results were no better with a gelatin medium. When an equal volume of platelet-poor plasma was added to Triton-saline, survival was definitely better, the index being 7 per cent after three days of storage. Best results were obtained with a plasma-Triton-saline medium to which had been added phosphate buffer, dextrose, inosine and adenosine. After three days of storage the viability index was 18 per cent; after six days 11 per cent.

This report suggests that storage of viable platelets for a limited period may become a practical procedure. Further work to define optimum conditions better is under way.—*H. A. P.*

Erythroblastosis

The negative antiglobulin test in Rh erythroblastosis. L. V. Crowley. U. of Vermont, College of Med., Burlington, Vt. Am. J. Clin. Path. 33: 496, 1960.

AN UNUSUAL case of severe erythroblastosis foetalis resulting from Rh incompatibility associated with a negative direct antiglobulin test is reported. Incomplete antibody was detected in the cord serum and demonstrated by both bovine albumin and antiglobulin technics. The cord erythrocytes readily typed "Rh positive" and after re-incubation with maternal serum, developed a positive direct Coombs test. The significance of these observations in the serologic evaluation of suspected erythroblastosis and the need for responsible clinical judgment in suspected neonatal hemolytic disease is apparent.—*A. S.*

The reliability of anti-human serums in the detection of ABO hemolytic disease of the newborn, by means of the direct antiglobulin test. S. Haberman, E. Aguilar, J. M. Solomon and J. T. Tripp. Baylor University Medical Center, Dallas, Texas, and National Institutes of Health, Bethesda, Maryland. Am. J. Clin. Path. 34: 179, 1960.

A SERIES of direct antiglobulin tests on cord blood samples from proven cases of ABO hemolytic disease of the newborn were performed employing different lots of anti-human serum. Three of the serum samples were selected at random from lots meeting the Minimum Requirements of the NIH and the fourth was one chosen for its known ability to react with antibody coated red cells of erythroblastic infants. Results of the tests varied among the four serums from negative to moderate positivity. The reactions with all the antisera were considerably enhanced when eluates prepared from the cord blood samples were used to sensitize adult erythrocytes.—*L. R. G.*

Red Cell Metabolism

Metabolism of the mammalian erythrocyte. I. M. London. Albert Einstein College of Medicine, New York. Bulletin of the New York Academy of Medicine 36: 79, 1960.

THIS IS an excellent and clearly presented review. The reticulocyte has an active Embden-Meyerhof glycolytic cycle, an active hexose-monophosphate pathway and an intact Krebs tricarboxylic acid cycle. A major constituent of the reticulum is RNA and its presence probably makes possible the formation of globin. The mature erythrocyte has little or no RNA and the tricarboxylic acid cycle is not intact. The mature erythrocyte derives its energy primarily from anaerobic glycolysis and from the oxidation of glucose via the hexosemonophosphate pathway. A principal task of the immature erythrocyte is the synthesis of hemoglobin. The complex molecule, heme, is synthesized from rather simple small molecule precursors, namely glycine and succinate, to delta-aminolevulinic acid. Two molecules of the latter condense to form porphobilinogen. The manner in which this is metabolized to ferrous protoporphyrin IX or heme is described. Much less is known about the synthesis of globin but a great deal of attention has been focused on it because of the great interest in the hemoglobinopathies. The manner in which hemoglobin is maintained in the reduced state is discussed. The importance of the age and the metabolism of the erythrocyte in determining its reactions is pointed out. Dr. London and his co-workers have shown that the young adult cells are far more resistant to immune lysis and as they begin to approach the end of their life span their susceptibility increases in a very marked fashion. In the case of osmotic hemolysis and primaquine-induced hemolysis it is the young cells, perhaps with the exception of the very youngest, which are more resistant to destruction. The metabolism of ATP is discussed at some length. It is known that the level of ATP declines progressively with concomitant loss of viability of erythrocytes which are stored in vitro and that in media in which the ATP content of the erythrocyte is maintained for a longer period the viability of the stored cells is prolonged. At any rate it seems to be agreed that ATP is required to maintain the integrity of the red cell in vivo and in vitro. The remarkable work of Alving, Beutler and Carson concerning the equilibrium between reduced and oxidized glutathione and the enzyme systems necessary to maintain this system is discussed. In closing there are raised the intriguing questions of whether the low level of reduced glutathione leads to the destruction of the red blood cell and if so what the mechanism of this hemolysis is or whether the changes in reduced glutathione and glucose-6-phosphate dehydrogenase accompany a more fundamental defect which is as yet undetermined.—*T. J. G.*

Changes in the phosphate compounds of the human red blood cell during blood bank storage. G. R. Bartlett and H. N. Barnet. Scripps Clinical and Research Foundation, La Jolla, California. J. Clin. Invest. 39: 56, 1960.

Phosphate compounds of the rabbit red blood cell during storage in acid citrate dextrose (ACD) and ACD-inosine. G. R. Bartlett and A. W. Shafer. *Ibid.* 39: 62, 1960.

Effect of inosine on the post-transfusion survival of stored rabbit erythrocytes. *Ibid.* 39: 69, 1960.

RED BLOOD CELLS from A.C.D. human blood stored for various periods up to 62 days were analyzed for phosphate compounds by a technic involving column chromotography on ion-exchange resins. Within three hours after collection the adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (DPG) were slightly lower than in blood collected in heparin. Inorganic phosphorus (IP) was three times higher. Fructose diphosphate was absent in the ACD red cells, whereas it formed one third of the hexose diphosphate fraction of red cells from heparin blood. In the ACD blood red cells, DPG fell precipitously in the first 15 days of storage. ATP fell much more slowly. Glucose diphosphate was stable. IP rose very rapidly during the first two weeks.

Red blood cells of the rabbit appear to react about the same as those of the human to storage in ACD. Rabbit blood was stored for 6 weeks in ACD and in ACD to which inosine had been added (4 mg. per ml. blood) (ACDI). 2,3-diphosphoglycerate and adenosine triphosphate fell slightly more slowly in ACDI than in ACD. Inorganic phosphorus rose less rapidly in ACDI. Adenosine diphosphate (ADP) showed only a slight drop at three weeks but had almost disappeared at six. Inosine slowed the breakdown of ADP somewhat. Fructose diphosphate was absent in the red cells of ACDI blood as it was in the ACD blood. Inosinic acid and sedoheptulose-7-phosphate increased strikingly with ACDI, but not with ACD.

The 24 hour post-transfusion survival of rabbit red blood cells from blood stored for six weeks in ACD was compared with that to which inosine had been added (ACDI). There was marked variability in the results between individual animals, but the ACDI red cells had a mean viability of 61 per cent compared to 18 per cent with ACD red cells. There was little difference whether inosine was added at the beginning of storage, after 21 days, or twice a week during storage. This report thus provides further evidence that inosine improves the proportion of viable red blood cells on prolonged storage in ACD.—*H. A. P.*

Miscellaneous

Further observations on leukoagglutinin transfusion reactions. R. Payne and M. Rolfs. Stanford University School of Medicine, Palo Alto, Calif. Am. J. Med. 29: 449, 1960.

THE MAJORITY of serums from 221 patients with febrile transfusion reactions contained leukoagglutinins. Women comprised a relatively large proportion of the persons with leukoagglutinin transfusion reactions and this occurred in many women after fewer transfusions than in men.

Half the women had received only one or two transfusion stimulations. In this group there was a high incidence of women with previous pregnancies. Leukoagglutinins for the white cells of husband and children were usually demonstrated. Non-parous women required more transfusion stimulations than parous ones. These observations suggest that leukoagglutinins probably develop from stimulation by fetal leukocytes.

Using any one of three methods, leukoagglutinin transfusion reactions were consistently prevented by the administration of blood from which the majority of the leukocytes had been removed.—*J. B. R.*

The effect of some mercaptanes upon a macrocryogelglobulin; modifications induced by cysteamine, penicillamine and penicillin. S. E. Ritzmann, S. L. Coleman and W. C. Levin. U. of Texas, Med. Branch, Galveston, Texas. J.C.I. 39: 1320, 1960.

SINCE mercaptanes have previously been demonstrated to depolymerize some macroglobulins from 18 – 32S to 6 – 7S components, a patient with Waldenström's macroglobulinemia was studied. The oral administration of dl-penicillamine and intravenous penicillin G produced a significant decrease of relative serum viscosity, a negative Sia test and an increase of erythrocyte sedimentation rate. The coincident administration of Benemid® with penicillin enhanced this effect and delayed a rebound of viscosity that was otherwise noted.

The in vitro addition of dl-penicillamine, penicillin G and cysteamine to serum decreased the relative viscosity, widened the macroglob-

ulin fraction in the electrophoretic pattern, abolished gel formation and produced a negative Sia test.

Agar diffusion studies revealed the simultaneous disappearance of the macroglobulin precipitin line and a quantitative increase in the gamma globulin line with dl-penicillamine and cysteamine but no change with penicillin G. Possible clinical implications are mentioned. —*A. S.*

Serum protein changes in Waldenström's macroglobulinemia during administration of a low molecular weight thiol (penicillamine). H. S. Bloch, A. Prasad, A. Anastasi and D. R. Briggs. U. of Minn. Hospitals, Minneapolis, Minn. J. Lab. & Clin. Med. 56: 212, 1960.

THE SULFHYDRYL concentration in sera of two patients with Waldenström's macroglobulinemia was found to be sub-normal. In vitro studies showed that the macroglobulin did not contain free sulfhydryl groups and depolymerization occurred with β,β-dimethylcysteine (dl-penicillamine). The oral administration of this drug to two patients with this syndrome was accompanied by a decrease of the macroglobulinemia but was without clinical benefit.—*A. S.*

Studies on the rheumatoid and related serum factors. I. Autoimmunization of rabbits with gamma globulin. F. Milgrom and E. Witebsky. University of Buffalo School of Medicine, Buffalo, N. Y. J.A.M.A. 174: 56, 1960.

RABBITS were injected either with their own gamma globulin prepared by ammonium sulfate precipitation and incorporated into Freund's adjuvant or with alum precipitated whole serum. Antibodies were produced which reacted with the gamma globulins of foreign species, especially with human gamma globulin, in test systems that involved complement fixation, tanned cell hemagglutination, and "anti-Rh coated" red cells. The reactions were easily inhibited by the addition of human gamma globulin or whole human serum, but they were not inhibited by the addition of rabbit gamma globulin. The reactions of these anti-sera were thus identical to the reactions produced by standard Coombs sera (rabbit anti-human sera). In addition, no differences between these sera and rabbit anti-human gamma globulin sera were detectable by immunoelectrophoresis or Ouchterlony agar diffusion methods.

Only insignificant in vitro reactions were obtained when rabbit material rather than human material was used as antigen. The concentration of rabbit gamma globulin had to be 1,000 times stronger than that of human gamma globulin to produce detectable reactions.

To explain the experimental findings the authors assume that the chemical procedures involved in the isolation of rabbit gamma globulin induce structural changes in a small proportion of the gamma globulin molecules. These altered molecules lose their original species specificity and assume a new antigenicity closely resembling that of human gamma globulin. The authors suggest the possibility that the rheumatoid factors in human sera may result from antigenic stimulation with the patient's own altered gamma globulins.—*H. F.*

Further modification of the benzidine method for measurement of hemoglobin in plasma. G. E. Hanks, M. Cassell and H. Chaplin, Jr. Washington U. School of Med., St. Louis, Mo. J. Lab. & Clin. Med. 56: 486, 1960.

A MODIFIED benzidine method is described in detail, with special emphasis on the relative stability of the various reagents and the accuracy of the method for the measurement of concentrations of hemoglobin in the range of 0.16 – 0.58 mg. per 100 ml. This was found to be the normal plasma hemoglobin range (mean 0.31 mg. per 100 ml.) in 15 ambulatory and 10 sleeping normal subjects. Observation of a normal resting subject for two hours showed no essential fluctuation in the plasma hemoglobin concentration. It is therefore estimated that less than 10 per cent of the normal destruction of red cells occurs by release of hemoglobin directly into the circulating plasma. —*A. S.*

The blood donor: I. Who are our blood donors? An analysis of social and other characteristics of 12,759 donors. F. F. Korzekwa, W. Q. Jordan and J. B. Alsever. Southwest Blood Banks, Scottsdale, Arizona. Am. J. Med. Sci. 240: 36, 1960.

II. Blood donors associated with homologous serum hepatitis. An analysis of 211 donors involved in 70 cases. J. B. Alsever. *Ibid.* 48.

THE SOUTHWEST BLOOD BANKS' chain of banks draws the majority of its blood, about 60 per cent, from paid (as opposed to replacement) donors. Inferences that the blood of the paid

donor might be less safe than that of the replacement donor led to a survey of the two types. Paid donors receive $10 if they are called, $5 if they spontaneously appear at the blood bank.

The survey disclosed a number of things: There were higher percentages of permanent residents and employed persons among the replacement donors. Most replacement donors donated for the first time. Paid donors are less likely to donate unsuitable units of blood, i.e., the incidences of positive serologies and of "short" bleedings were both lower in this group. Paid donors are more likely to return for future donations: this suggests that replacement donors are somewhat "unwilling" to donate. The hepatitis carrier rate seemed to be the same in the two groups. However, there was a strikingly high incidence of hepatitis carriers among new or one-time donors. This involves replacement donors relatively more significantly in that replacements are more apt to be new or one-time donors. Some of these data have led the authors to prefer paid donors.

The data further validated the conclusion that hepatitis suspect donors in multiple transfusion cases should be rejected unless there is a record of two "safe" previous donations in addition to other negative findings.—*J. B. R.*

Plasma thromboplastin component (Christmas Factor, Factor IX) levels in stored human blood and plasma. J. D. Geratz and J. B. Graham. University of North Carolina, Chapel Hill, North Carolina. Thrombosis et Diathesis Haemorrhagica IV, No. 3/4, 376, 1960.

AFTER WHOLE BLOODS were stored at 4 C. for 19-25 days, the plasmas were removed and stored at −20 C. for periods up to 114 days. Factor IX assays then showed levels varying from 55 per cent to 208 per cent of normal, with a mean of 95 per cent. There was no evidence of progressive loss during this period of storage at −20 C.; but an additional six months' storage caused a loss of Factor IX activity averaging 23 per cent. After four years at −20 C., the Factor IX level was 68 per cent. Liquid plasma after six months at room temperature had lost all activity. Lyophilized plasma at room temperature for 6-8 years assayed at 30 per cent. Results were the same whether the blood was collected in ACD or disodium hydrogen citrate.

Plasma prepared from out-dated bank blood and further stored at −20 C. was administered to a Factor IX deficient patient undergoing dental surgery. Clinical response and assays of his factor IX levels showed that the Factor IX in these stored plasmas had the expected beneficial effect.

It is apparent that patients with Factor IX deficiency can be adequately treated with plasma salvaged from out-dated bank blood.—*H. A. P.*

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This Section and Sections for Short Communications and Questions and Answers will be presented as material becomes available.

Technologists are invited to submit manuscripts which will be given careful consideration for publication.

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Legal Problems:

Blood Banking and the Implied Warranty*

HOWARD HASSARD
San Francisco, California

THE LEGAL ASPECTS of blood banking are still in a relatively early stage, and to my knowledge, as yet there have been no cases *directly* involving community blood banks which have reached an appeal court. However, in recent years a few cases involving *hospitals* and their liability with respect to blood transfusions have been reviewed by appellate courts, and the law enunciated therein would also be applicable to blood banks.

Today's society being as complex as it is, almost every business or professional problem is to a certain extent a legal problem. Hence, a discussion of all of the legal problems of blood banking would necessarily include an extensive discourse on a multitude of legal obligations to which a blood bank is subjected from the matter of organization of a blood bank to statutory law regulating the operation of blood banks in any given state. Time, of course, precludes such a discussion. The problems arising from the relation of the blood bank to the donor have been, I am certain, covered elsewhere. As you know, it is necessary to obtain a proper consent from each donor because the taking of blood in the absence of such a consent constitutes a technical assault and battery just as does any surgery or any injection. It is therefore important that blood should under no circumstances be taken without the consent of the donor or one having authority to give a valid and binding consent for him, and, to avoid the unpleasant consequences of a civil suit for an unauthorized battery, that the consent be clearly expressed in writing and preserved by the bank.

Once the consent of the donor has been secured, the blood bank then has the duty to exercise reasonable care in obtaining the blood donation and in properly caring for the donor during the course of a blood donation. The term "reasonable care" would in the case of blood banks, as in the case of physicians, be a relative standard, that is, one which would be established by a course of conduct followed by competent and careful blood banks operating in the same general locality. Any conduct falling below such a standard would, of course, constitute negligence, for which the blood bank would be held liable to the donor. In this connection also, a blood bank is liable to the recipient of blood, that is, the patient, for the damages which result from its negligent acts, such as negligent mislabeling of blood types and negligent processing whereby an infection which could have been detected and guarded against is transmitted to the patient because the bank failed to make the proper tests, or where tests

* Read before the 13th Annual Meeting of the American Association of Blood Banks, San Francisco, California, August 21, 1960.

were improperly conducted. To protect fully against liability for negligence requires, of course, the exercise of the utmost care and vigilance on the part of the blood bank and its employees and the maintenance of rigid testing standards.

Because time will not permit a discussion of all of the major legal problems confronting blood banks, I have elected to devote the remainder of my time to a problem of current interest, and one, which I might add, is potentially much more serious, to blood banking, that is, the problem that exists with respect to serum hepatitis and the legal "animal" connected therewith, the implied warranty.

Before embarking on a discussion of implied warranty and its relation to the transmission of serum hepatitis in blood transfusions, I should briefly point out that it seems clear that as long as there is no known test for detecting the presence of hepatitis virus in blood or plasma, there can be no recovery on the theory of negligence since the exercise of the highest standards and the most rigid testing procedures simply do not help under such circumstances. This was reaffirmed in a very recent case, *Fischer v. Wilmington General Hospital* (149 A. 2d 749) decided by the Supreme Court of Delaware, in which a patient sued a hospital on the basis of negligence on the part of the hospital's agents in administering to her blood containing the causative agent of serum hepatitis disease. The court in affirming a judgment for the defendant hospital pointed out that there is no known medical technic by which the virus which causes hepatitis can be detected or destroyed in the blood, and that the blood used in the transfusion involved was procured under conditions which imposed all reasonable safeguards to assure that it did not contain the virus causing hepatitis. Therefore, so long as medicine has not devised a test to discover the presence of hepatitis virus, there can be little doubt that no court can possibly reach the conclusion that the distribution of infected blood constitutes negligence *per se*.

Now, to the problem of implied warranty. The law of warranty was developed to protect purchasers and has been embodied in the Uniform Sales Act adopted throughout the United States. What is meant by an implied warranty? Generally it means that when there is a sale of goods, the seller, without making any express representations himself, implicitly warrants that the goods will at least be fit for the purpose for which they are ordinarily used. If the goods are not fit, then the seller is liable for any damages that occur, including compensation for personal injury. Note that the seller in such a case is liable even in the absence of any fault or wrongdoing on his part. However, the law also states that there can be no warranty without a sale.

Because of the difficulties in recovering on a negligence theory in serum hepatitis cases, an attempt was made by plaintiffs contracting the disease as a result of a blood transfusion to recover on an implied warranty theory, that is, to impose liability without fault. The first of these cases to reach the appellate level was the New York case of *Perlmutter v. Beth David Hospital* (123 N.E. 2d 792), decided in 1954. In that case, suit was instigated for damages resulting from jaundice claimed to have been received through a blood transfusion, and was brought against the hospital on the theory that the supplying of blood constituted a sale and was therefore subject to an implied warranty that the blood was reasonably fit for the purpose for which it was required, and of merchantable quality. The highest Court of the State of New York in a very close decision (4-3) held for the defendant hospital, refusing to imply a warranty except in conjunction with a technical sale. The court found that in a transfusion the transfer of blood is incidental to the service rendered, and since service predominates, the transfer of the blood is not a sale, and therefore no implied warranty attaches.

In 1956, the Supreme Court of the State of Washington was faced with the same problem in a suit against a hospital based on the theory that the blood used in the transfusion was sold to the patient and that there was a warranty of fitness for the purpose intended. The Court in *Gile v. Kennewick Public Hospital* (296 P. 2d 662), cited the Perlmutter case with approval, holding that the transfer of blood by a hospital to a patient used in a routine blood transfusion was not a "sale," and therefore, there could be no recovery for alleged breach of warranty of fitness.

Although the two cases referred to involve suits against hospitals, let me state that the question of liability for the transmission of hepatitis does not concern hospitals alone. It is true that if the courts do allow recovery on the implied warranty theory, the patient's suit will generally be against the hospital and not the blood bank since in the majority of jurisdictions the general rule is that a manufacturer or seller is not liable under an implied warranty to third persons who have not purchased directly from him, but liability rests only on

warranty to the person to whom the sale was made. This, however, does not permanently insulate blood banks from liability since a hospital upon whom liability has been imposed on an implied warranty may in turn recover from the blood bank supplying it with blood.

In California, there are no appellate cases involving implied warranty and its application to a transfer of blood. However, in 1955 Section 1623 was added to the Health and Safety Code. This code section declares the procurement, processing and distribution of whole blood, plasma and blood products for purposes of transfusion or injection to be a rendition of a service and not a sale. The statute has not been challenged as yet on constitutional grounds, and presently precludes an implied warranty from attaching to a transfer of blood for the purposes described by its terms and will continue to do so unless overturned by the courts. However, it is interesting to note that two years prior to the enactment of the statute, a suit was brought here in San Francisco by a plaintiff who allegedly contracted serum hepatitis subsequent to blood transfusions of whole blood and plasma. The action was prosecuted against the blood bank furnishing the whole blood, the drug company distributing the plasma and against the hospital on an implied warranty theory. The blood bank was voluntarily dismissed, and the Trial Judge directed a verdict for the remaining defendants, ruling that there was no sale, but a rendition of services, and thus no implied warranty attached (*Gomberg v. Ohio Chemical & Mfg. Co., et. al.*, No. 403171).

The outstanding fact about the cases I have heretofore discussed is that the courts have refused without exception to imply a warranty in suits for damages resulting from serum hepatitis claimed to have been received through a blood transfusion. However, we must be realistic and recognize that few cases involving this question have been reported, and in addition, the Perlmutter case, which is the leading case, was a very close decision. On the other hand, when the question is presented for appellate review in other states, a contrary holding may result. Until a sufficient number of cases arises to indicate a pattern of decisions, the application of the doctrine of implied warranty to blood banking will not become clear. If the courts abandon the theory of the Perlmutter case, the blood banks will of necessity be compelled to seek other solutions, such as a disclaimer of implied warranty of fitness.

Within the past few weeks one of the California District Courts of Appeal has applied the doctrine of implied warranty against Cutter Laboratories, the manufacturer of Salk vaccine that proved faulty in that it contained live virus causing several vaccine recipients to contract poliomyelitis. The California Supreme Court has denied a hearing in this case, thus the decision has become law in this state. This is the first time that an appellate court in the United States has applied the doctrine of implied warranty to biological or pharmaceutical products. The doctrine was imposed against the manufacturer, even though there was *no* actual sale of the vaccine to the recipients—in each instance it was administered by a physician who simply charged a service fee. It is entirely too soon to draw any definite conclusion from the Cutter cases; except that once again the national trend toward compensation for personal injuries, irrespective of fault, is noted.

Of course, vaccine is not blood, and the fact that a warranty was implied against Cutter Laboratories does not mean that in the future courts will change their attitude in cases involving transfusions.

The essential question to be determined is upon whom is the loss ultimately to fall when, without fault on the part of anyone, a recipient of blood contracts hepatitis and suffers damage as a result. Is it to be the blood bank which collected the blood and distributed it to the hospital, or should it be the hospital furnishing the blood to the patient, or the patient himself? As we have seen, the courts thus far held that the loss should fall on the patient, but the future is far from clear.

Correspondence

To The Editor:

THERE is no National Blood Program, and yet, there are many National Blood Programs. This seeming paradox is self evident to anyone who has the opportunity to study the reports of various agencies and organizations that concern themselves with blood. Because of a rather chaotic state of affairs regarding the definition of the term "National Blood Program," I was prompted several months ago to ask this question of a number of people who are or should be concerned with it.

A letter was sent to 33 people asking them to give their concept of the term "National Blood Program." This letter went to officials of the Federal Government, to officials of quasi-governmental agencies and officers of various professional organizations concerned in this problem. Response to my letter was very gratifying and I am most appreciative of the time and thought that was spent writing replies to my question.

There is unanimity of expression from agencies of the Federal Government. This is based on a Presidential Directive of 1951, in which the following sentence appears: "The program created to meet the nation's blood needs during the national emergency shall be called 'The National Blood Program.'" A clear and concise statement of their position is given by Dr. Frank Berry, Assistant Secretary of Defense, and I will quote it here: "As defined in the 'Statement of Basic Principles' issued by the Office of Defense Mobilization and the Executive Office of the President, on 3 June 1957, the 'National Blood Program' is a national emergency activity. It is designed to fulfill the nation's needs for blood and related substances during a national emergency. As such it is not in complete operation during peace time. Peace time operations are limited to:

A. Planning and coordination thereof by and among all responsible agencies and organizations.

B. Maintenance of stock piles of blood, blood derivatives, synthetic expanders and related supplies and equipment.

C. Maintenance of stand-by facilities for blood collection, processing, and shipment; and the manufacture of derivatives and expanders.

D. Training of professional and technical personnel.

E. Conduct of related research and development."

This is the position of the governmental agencies on the National Blood Program. It is clear, concise and not given to misinterpretation. The American Red Cross has accepted this definition and General Gruenther further pointed out that following the President's Directive of 1951, the name of the Red Cross Program was changed in April, 1952, from "National Blood Program" to "The Red Cross Blood Program."

From this, one can see that the definition of a "National Blood Program" is a matter of simplicity, and I really was wasting my time to ask a question with such a simple answer. But, this is not the whole story, and I would suggest that we now "step through the looking glass" and look at other facets of the problem. Where there was unanimity of thought, there is now a great variance of opinion as to what is meant by our term "National Blood Program." It would seem that aside from the governmental agencies, where thought has been crystalized by the Presidential Directive, everyone has a different idea as to exactly what is meant by this term. I would now like to examine some of these opinions and to boldly suggest a standardized nomenclature so that all may know whereof we speak. I realize that my chances of success in this endeavor are probably as futile as agreement in other phases of this field of blood banking.

The concept of a National Blood Program varies from one extreme to another. On the one hand, there are those who think the ideal program is one in which all phases of blood transfusion services should be the responsibility of an organization, governmental or otherwise, which would operate this program on a broad national basis. On the other, there are those who feel that a National Blood Program should be one in which the local unit is autonomous with as little outside direction as possible, but with some system of reciprocity of "blood credits" on a national basis. All agree that in time of national emergency, the coordination of existing facilities for the provision of blood and blood products for all the people is properly a function of a governmental agency in a supervisory capacity.

There are two principal reasons for the great variation in opinions as to the concept of a National Blood Program. These are (1) the establishment of the Joint Blood Council and (2) the recent interest of certain organizations not directly concerned with blood banking in providing blood for *all* persons of the United States. The Joint Blood Council was established by five member organizations, all of whom were more or less directly concerned with blood banking activities in the United States. In the Articles of Incorporation of the Joint Blood Council, one of the purposes of the organization was stated as follows: "To develop ways and means to make blood and its derivatives available to all persons in the United States." The interpretation of this particular phase has been a source of disagreement even within the Joint Blood Council. However, two of the member institutions have recently affirmed the original proposition that the Joint Blood Council was not to be an "operative organization" and this view has been further expressed by another professional organization with interests very closely allied to these associations. This opinion concerning the "operation" of the Joint Blood Council has also been expressed by one of the past Presidents of the Council. On the other hand, there have been those who feel that the Joint Blood Council should engage in an active operative program in the field of blood banking. An opinion as to the merits of these points of view is not pertinent to this discussion.

In addition to all this, another factor has appeared which adds further confusion to the picture. This is the interest which has been expressed in the past few years by various organizations to provide a mechanism by which all members of these organizations will be assured of obtaining necessary blood and blood derivatives in time of need. There are a number of these programs, all with different objectives. The most recent, and probably the most far reaching, is that of the AFL-CIO Community Service Activity Committee. Mr. Leo Perlis, the Director, stated in a letter December 30, 1959, "Our new program priority for 1960 will be blood banks," and the AFL-CIO in its recent convention passed a resolution on blood banking which states that this convention "affirms the need for a national, uniform, voluntary system of blood banks." There are also other organizations which have expressed concern in this field; for instance, the Veterans of Foreign Wars.

It can be seen that there are a great number of divergent ideas concerning the relatively simple matter of defining a "National Blood Program" and, therefore, I would like to make the following proposals as a guide to nomenclature in this field: (1) that the term "National Blood Program" be confined to that program which is based on the Presidential Directive of 1951, and is a program created to meet the nation's blood needs during a national emergency. (2) That any other program designated as a "National Blood Program" carry after the words "National Blood Program," in parentheses, the name of the author or organization proposing such a program, e.g., National Blood Program (Joint Blood Council), or National Blood Program (American National Red Cross), or National Blood Program (John Doe). This simple device will do two things: (1) it will give us a definite and understandable definition as to what is meant by "National Blood Program," and (2) it will immediately identify any other proposed country-wide program as to its author and its content, so that in speaking of these various schemes, we shall at once know the content and the purpose from its designation.

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A Guide for Preparation of Abstracts

Contributors are requested to prepare abstracts of their articles and submit them immediately before the body of the paper. Thus, summaries will not be necessary.

CONTENT—Include:

1. Name of organism, and objective of the study.
2. Materials, manner of use, or route of administration, principal findings, and results.
3. New technics, their uses and qualities.
4. New apparatus, its intended use, and if commercially available, name and address of its manufacturer.
5. New or verified data of permanent value, e.g., absorption spectra, chromosome numbers, constants, mathematical or chemical formulae.
6. New genera of animals/plants, new classifications, new distribution records. (For systematic data, see below.)
7. New theories, new interpretations, evaluations, if possible; if not, reference to them.

—Omit:

1. Information contained in the title.
2. Additions, corrections, or any information not contained in the original published paper.
3. Tables and graphs.
4. Detailed descriptions of experiments or organisms.
5. Long lists of names; species or subgenus names without genus name. (See directions for systematic papers, below.)

FORM—1. Begin with a citation in the following form: DOE, JOHN J. (U. Commonwealth, Capitol City), and RICHARD ROE. Metabolism of phosphorus in rats. Jour. Pest Control Res. 37 (4): 152-165. 1957. (Foreign language titles should be followed by an English translation.)

2. Use abbreviations sparingly, and only as directed.* (See below.)

3. For chemicals, use standard rather than proprietary terms; avoid trade names.

4. For organisms, use genus and species names, always underlined, excepting widely used experimental species (dog, rabbit) and commonly cultivated crops (apple, potato). The name should be affiliated, either formally [Vespidae: Hymenoptera] or informally "a leguminous tree," or "a staphylinid beetle," either in the title or in the first sentence.

ABBREVIATIONS—Use sparingly. Consider the reader who is not a specialist, or to whom American English is a foreign language. When in doubt, spell it out.

Do abbreviate or symbolize:

1. Those units of weight and measure listed in the Handbook of Chemistry and Physics, 35th Edition 1953-1954, pages 3100-3102 (Chemical Rubber Publishing Co.), of which the commonest are listed below:* but only when preceded by numerical amounts. . . . . "10 μg" but "several micrograms." . . . . . "40%" but "per cent of gain."
2. Numbers, except at the beginning of a sentence.
3. Chemical elements, except when part of the name of a compound. "K deficiency" but "potassium 2-naphthyl sulfate excretion."
4. Names of institutions and journals, as carried in the BIOLOGICAL ABSTRACTS Journal List.
5. The words species, subspecies, variety, form, but only in taxonomic abstracts.
6. Substantives used repeatedly, such as names of compounds, hormones, genes, but only after they have been spelled out the first time used in each abstract, followed immediately by the symbol in parentheses, "luteinizing hormone (LH)" or "acetylcholine (ACh)" or crossveinless (cv)." Such symbol-letters should not be spaced, nor underlined. ACTH, not A C T H.

Do not abbreviate:

1. Geographical names.
2. Short words such as day, year, ton.
3. Any special technical terms, no matter how commonly used in your field, unless treated as in number 6 above.
4. Greek letters, except in chemical compounds.

*Abbreviation for both singular and plural, except as noted.

basal metabolic rate	BMR	lethal dose	LD_{50}	ounce	oz.
chemical elements	use symbols	minimum lethal dose	MLD	parts per million	ppm
cubic centimeter	cc	meter	m	per cent	%
cycles per second	cps	microgram	μg	per mille	‰
degrees of temperature	25° C or F	micron	μ	pound	lb.
electrocardiogram	ecg	milliampere	ma	respiratory quotient	RQ
electroencephalogram	eeg	milliequivalent	meq	species (for tax. only)	sp. (spp.)
form (for tax. only)	f.	milligram	mg	subcutaneous	subcut.
gram	g	milliliter	ml	subspecies (for tax. only)	ssp. (sspp.)
hydrogen ion concen.	pH	names of months	Jan., Feb.,	ultraviolet	UV
kilogram	kg		Aug., Sept., Oct., Nov., Dec.	variety (for tax. only)	var. (vars.)
kilovolt	kv	molar	M	volume	vol.
		normal (solution)	N		

FOR SYSTEMATIC PAPERS—Additional instructions.

FORM: 1. Write the name of a subgenus, genus, or any supergeneric taxon, if old, in small letters with a capital initial; if new, all in capitals. The name of a species, subspecies, variety, or form is never capitalized.

2. Always underline the name of a genus, subgenus, species, subspecies, variety, and form: with a straight line if old, with a wavy line if new. No other group name is ever underlined. Write the name of a new combination (e.g., transfer of a species from one genus to another) with a straight line under the old and a wavy line under the new or shifted part, followed by the basonym and its author in parentheses.

CONTENT: Be alert to information *other* than the formal, strictly taxonomic and nomenclatural. Summarize or mention new data in any biologic field—life history, morphology, biogeography, cytology, ecology, evolution. (Non-taxonomists welcome such information.)

NEW TAXA. Omit detailed descriptions, long synonymies and taxonomic history, but mention their presence. Mention individually, by name, new genera and supergeneric taxa except where this would require a long list, as in some monographic revisions; do not mention by name a long list of new species and subspecific taxa when these are merely proposed and described—give only number in each genus, naming genus and country or region of origin. (*Exception: always include new species names of bacteria and viruses.*)

PROPOSALS OF NEW TAXA. For plants: include name of genus, family, and phylum (divisio, Abteilung); country, state, or region; number of each kind of new taxon; type of critical evidence, e.g., floral morphology, distribution, chromosome number. For animals: as above, except: genus, family, and (for insects, order) for other groups, class and phylum. Thus: Sarcophagidae [Diptera], 00 new genera and 00 new spp. in U. S., from a Colorado mountain previously unexplored; or, *Graphops* [Chrysomelidae: Coleoptera], 10 new spp. and 43 new sspp. in Erewhon, on the basis of genitalic characters; or, *Tropicorbis* [Planorbidae: Gastropoda, Mollusca] *grandis*, 6 new sspp. in Brazil, based on shell morphology and capacity to transmit X disease; or *Poa* [Gramineae: Monocot.], 3 new spp. in Norway, on the basis of morphology, chromosome number, and ecologic amplitude.

PROPOSALS OF CHANGE IN CLASSIFICATIONS AND/OR NOMENCLATURE (e.g., new combinations, names) should include:

For transfers, or changes of rank: The proposed combination and, in parentheses, the basonym and its author. *Xylobium* **pickianum** (*Bifrenaria pickiana* Schlechter); or, *Oncidium brachyandrum* var. **johannis** (O.j. Schlechter) For changes of status (validity or synonymy) The valid name, its author if not new here and, in parentheses the invalid name and author, preceded by an = sign. Explanatory word or phrase is desirable, e.g., "revived" or "preoccupied." *Erisma floribundum* Rudge (= *E. parvifolium* Gleason), or, **GANELIUS** (= *Nagelius* Benesh preocc.)

DISTRIBUTION DATA should mention:

1. First records. *"Rheumatobates clanis, R. minutus,* and . . . . . are new to Florida and to the U. S."

2. New localities, range statements, distribution maps. "New records (listed) for *Eurycea lucifuga* show that it does not have a disjunct range in Illinois, but extends . . ."

3. Range changes. "Species representing major extensions of geographic range in the Canadian Arctic Archipelago are: *Aulacomnium acuminatum,* . . ."

4. Faunistic/floristic changes, e.g., establishment and naturalization of weed spp.; spp. approaching extinction.

5. For fossils, epoch or period for each formation or group mentioned.

SAMPLE ABSTRACTS

TRAVIS, JOHN W., ALEXANDER C. KEYL, and CARL A. DRAGSTEDT. (Northwestern U. Med. Sch., Chicago.) *The effect of pancreatectomy on the toxicity of k-strophanthin in the dog.* J. Pharmacol. and Exptl. Therap. 117 (2): 148-150. 1956.—Comparison of the lethal doses of k-strophanthin in normal and depancreatized dogs indicates that the diabetic state raises the resistance of the animals to the toxic effects of the drug as determined by the intravenous lethal dose procedure. The lethal doses for 14 normal and 8 depancreatized dogs were 0.147 and 0.214 mg/kg, respectively, which is a significant difference at the 1% probability level. Animals rendered hyperglycemic by intravenous administration of d-glucose are not afforded similar protection, and these results tend to support the belief that insulin is involved in the transport of k-strophanthin across the cell membrane.—A. C. Keyl.

TRYON, ROLLA M., Jr. *Selaginella rupestris and its allies.* Ann. Missouri Bot. Garden 42 (1): 1-100. Illus. 1955.—A monograph of the section Tetragonostachys of the subgenus *Selaginella* (43 spp.); with taxonomic history, morphology and life history, phylogeny, distribution and ecology preceding the systematic treatment. Included are a synopsis of the subgenus, proposals of 2 sections with 4 new series, 4 new spp. and 4 new sspp., descriptions, keys, literature and specimen citations, synonymies, maps, and photographs. Cited as of special interest are the xeric ecology of the group (prolonged dormancy on desiccation), and the evolutionary significance of the distribution patterns of its sexual and apogamous races.

DILLON, LAWRENCE S. (A. and M. Coll. Texas, College Station.) *The neotropic Acanthocinini (Coleoptera: Cerambycidae). II. A further note on Canidia allies.* Ent. News 67 (4): 105-107. 1956.—*Pseudocanidia* **ochreosticticus,** Michoacan, Mexico.—J. L. Williams.